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THE EFFECTS OF AMPHENONE ON THYROID FUNCTION IN MAN*†

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AMPHENONE is a synthetic chemical compound of particular biologic significance because of its diverse endocrine properties. It was selected for study by Hertz and his associates as a result of its structural similarity to stilbestrol. The chemical formula originally assigned to amphenone by Allen and Corwin (1) was that of a desoxybenzoin derivative (Fig. 1A). This has recently been modified by Allen and Bencze (2). The new structural formula (Fig. 1B) is that of a substituted diphenylmethane compound. This alteration in the designated molecular configuration of amphenone is of practical importance in helping to define the relationship between chemical structure and biologic activity necessary for the selection of analogous

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compounds for further investigation. It should be noted that the new formula assigned to amphenone is similar to that of the adrenocorticolytic insecticide, 2,2-bis-(p-chlorphenyl)-1,1-dichlorethane, known as "DDD" (Fig. 1C) (3–5).

The following biologic properties of amphenone have been demonstrated in animals: (a) an atypical folliculoid response of the genital tract of rats (6); (b) a weak progestational action on the rabbit uterus (7); and (c) simultaneous enlargement of the thyroid and adrenal glands of rats (6, 8, 9). The goitrogenic effect of amphenone in rats can be inhibited by the administration of thyroid hormones without modifying its effect in causing adrenal enlargement; and conversely, adrenal enlargement is prevented by

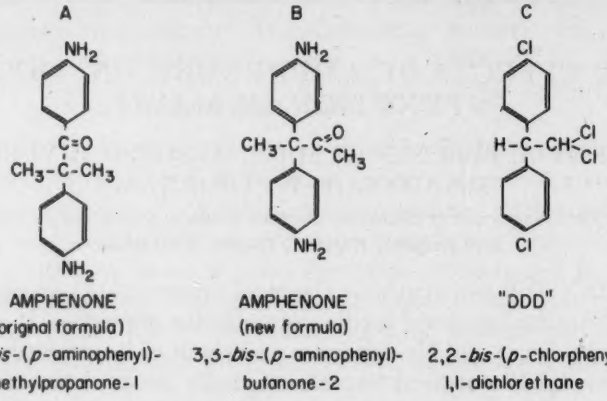

FIG. 1. (A) Original formula for amphenone. (B) New formula for amphenone. (C) Structural formula for "DDD."

administration of cortisone without interfering with goitrogenesis (10). The observation that neither goitrogenesis nor adrenal enlargement occurs in hypophysectomized animals receiving amphenone (7) supports the hypothesis that the thyroid and adrenal enlargement result from pituitary stimulation. On the other hand, the effects on the genital organs persist in both hypophysectomized and adrenalectomized animals, indicating a direct action on these structures. In hypophysectomized dogs, the administration of amphenone suppresses the secretion of 17-hydroxycorticoids following adrenal stimulation by corticotropin (ACTH), as measured by the method of Nelson and Hume (11, 12). A direct inhibitory effect of amphenone has also been demonstrated *in vitro* by perfusion studies with beef adrenal glands (13). The various biologic properties of amphenone in animals have recently been reviewed by Hertz and his associates (14).

The goitrogenic effect produced in animals by amphenone is well substantiated (6, 8, 9, 10, 15–17); this action of amphenone is consistent with the established goitrogenicity of numerous aminobenzene derivatives (18) including certain of the hypoglycemic arylsulfonylureas (19). Preliminary studies have indicated that amphenone blocks the incorporation of radioactive iodine into the thyroid gland both *in vitro* and *in vivo* and that it apparently lacks an effect on the rate of release of I^{131} from the gland (8). Recent studies in cockerels have further demonstrated that the action of amphenone on the thyroid gland is similar to that of the thiourea group of goitrogens, in that organic "binding" rather than accumulation ("trapping") is inhibited (16); the relative potency of amphenone in this regard is less than that of compounds of thiourea type (17).

In man, amphenone likewise exerts rather striking effects on adrenal and thyroid function (20). In subjects with normal adrenal function, administration of amphenone produces a definite, but slight, suppression of blood and urinary levels of 17-hydroxycorticoids (20, 21) as well as urinary 17-ketosteroids (20, 22) and aldosterone (23). Further, amphenone inhibits adrenal steroidogenesis in patients with hyperadrenocorticism (20, 24) and functioning adrenal carcinoma (20, 25, 26).

Hertz, Pittman and Graff (21) recently reported the effects of multiple doses of amphenone on the thyroid function of euthyroid and hyperthyroid patients. The present study was undertaken to determine the action of both single and multiple doses of amphenone on thyroidal "trapping" and "binding" of radioactive iodine in euthyroid and hyperthyroid subjects.

METHODS AND MATERIALS

The amphenone used in this study was kindly supplied by Dr. Roy Hertz of the National Institutes of Health and was administered by mouth in the form of gelatin capsules, each containing 250 mg. of amphenone as the dihydrochloride. Radioactive iodine uptake studies were performed utilizing a scaler (Nuclear-Chicago) equipped with a scintillation counter (Nancy Woods). Tracer curves were obtained by plotting serial determinations of external thyroidal counts taken at indicated intervals following the oral administration of 10 to 15 microcuries of I^{131}; appropriate corrections were made for physical decay, back scatter and residual radioactive iodine activity, if present. Potassium thiocyanate was administered orally in a simple syrup containing 1.0 Gm. of KSCN in each 10 ml. of solution. The trade name Tapazole® is employed in this report as synonymous with the U.S.P. designation methimazole (1-methyl-2-mercaptoimidazole).

Most of the patients studied were hospitalized on the metabolic ward of the Peter Bent Brigham Hospital; a few were studied as out-patients. Clinical diagnoses of euthyroidism and hyperthyroidism were confirmed by determinations of the 24-hour uptake of radioactive iodine, serum protein-bound iodine and cholesterol levels, and basal metabolic rate.

RESULTS AND DISCUSSION

Six euthyroid subjects were studied. The administration of amphenone in single doses of 0.25 and 1.0 Gm. one hour prior to the tracer dose of radioactive iodine induced a 50 to 75 per cent reduction in the 24-hour thyroidal radioactive iodine (I^{131}) uptake (Fig. 2). A dose-response relationship was noted. Serial daily fractional doses totaling 4.0 to 8.0 Gm. of amphenone caused complete suppression of the I^{131} uptake, as measured by external

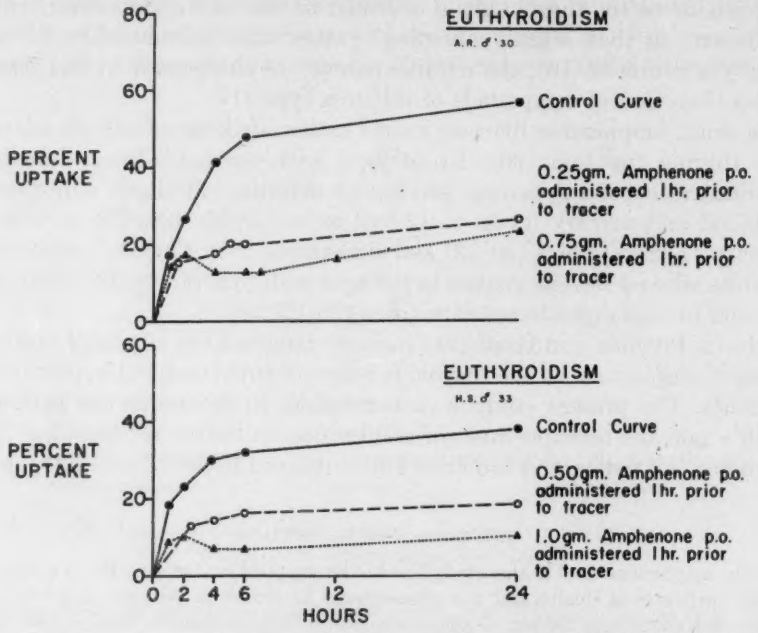

FIG. 2. The effect of various single small oral doses of amphenone administered one hour prior to the I^{131} tracer test in 2 euthyroid subjects.

counting. With 4.0 Gm. or less, the four-hour and six-hour readings occasionally reflected the presence of small quantities of radioactive iodine, but by twenty-four hours this had apparently been released from the thyroid gland (Fig. 3). Following discontinuance of amphenone, thyroid function usually returned to normal within forty-eight to seventy-two hours, indicating complete reversion of the amphenone block. This is in accord with the known rapid excretion of the drug.

In 11 hyperthyroid subjects, administration of 0.5 to 1.0 Gm. of amphenone by mouth one hour prior to initiation of the I^{131} tracer curve likewise induced a 50 to 75 per cent suppression of the 24-hour thyroidal I^{131} uptake

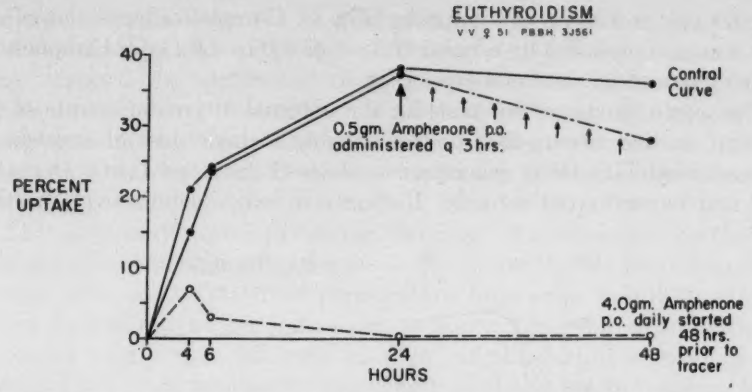

FIG. 3. The effect of small oral fractional doses of amphenone administered daily on the 4-hour, 6-hour and 24-hour thyroidal I^{131} uptake of a euthyroid subject.

(Fig. 4). A dose-response relationship was again noted until complete thyroidal suppression of radioactive iodine uptake occurred. The inhibitory effect of amphenone disappeared by seventy-two hours, as indicated by a

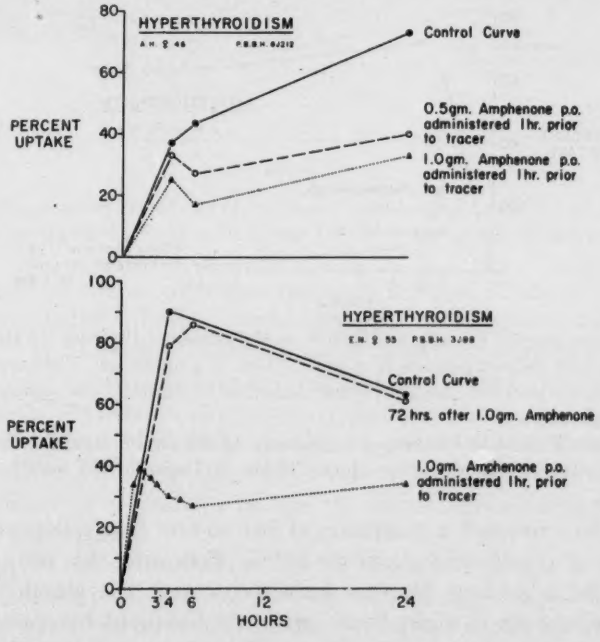

FIG. 4. The effect of various single small oral doses of amphenone administered one hour prior to the I^{131} tracer test in 2 hyperthyroid subjects.

return to control levels of I^{131} uptake (Fig. 4). Complete suppression of uptake was accomplished by administration of 4.0 to 8.0 Gm. of amphenone daily by mouth in fractional doses (Fig. 5).

The curve produced by plotting the external thyroidal counts of I^{131} at four, six and twenty-four hours following a single dose of amphenone seemed to exhibit a fairly characteristic shape (Figs. 2 and 4) in both euthyroid and hyperthyroid subjects. Radioactive iodine counts over the thy-

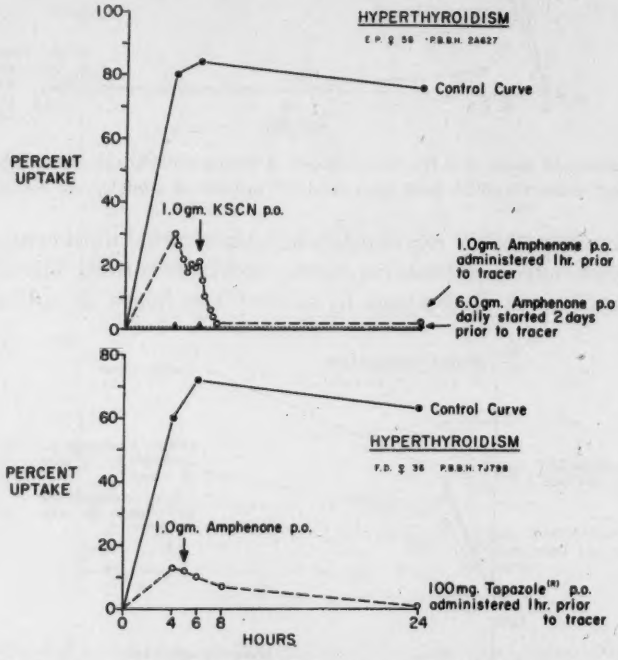

FIG. 5. *Upper graph:* The affect of KSCN on the release of I^{131} from the thyroid gland of a hyperthyroid subject previously given 1.0 Gm. of amphenone. The rapid discharge of I^{131} from the amphenone-blocked thyroid indicates an inhibition of organic "binding" of I^{131} by amphenone.

Lower graph: Failure of 1.0 Gm. of amphenone to release I^{131} from the thyroid gland of a hyperthyroid patient previously given 100 mg. of Tapazole® by mouth.

roid gland rose to reach a maximum at two to four hours, depending upon the avidity of the thyroid gland for iodine. Following this peak there appeared to be a gradual decrease in activity over the gland, forming a trough at about six to eight hours, with a subsequent reaccumulation of radioactive iodine at twenty-four hours. However, with larger doses, this second rise at twenty-four hours was not present (Fig. 3). The release of

radioactive iodine from the thyroid gland after amphenone inhibition is best explained by a loss of "unbound," inorganic iodide to the plasma (27). The "trapped" or "unbound" thyroidal radio-iodide results from the inhibition of organic "binding" by amphenone—that is, a failure to form mono- and diiodotyrosine. As the effects of amphenone inhibition decrease, some of the plasma radioactive iodine appears to be reaccumulated and becomes organically bound.

That amphenone inhibits organic "binding" of radio-iodide by the thyroid gland is demonstrated in Figure 5. This hyperthyroid patient received a single dose of 1.0 Gm. of amphenone one hour prior to initiation of the tracer dose of radioactive iodine. At six hours, when partial inhibition of thyroidal uptake was definitely manifest, administration of a single oral dose of 1.0 Gm. of potassium thiocyanate produced a rapid and complete

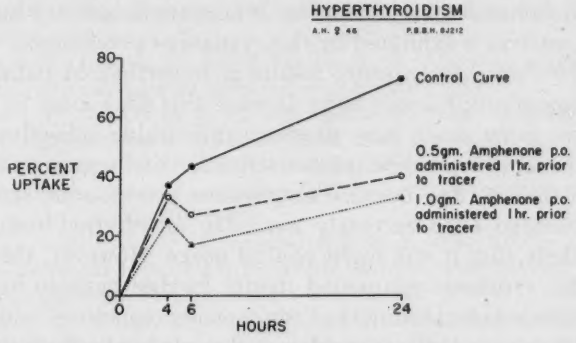

FIG. 6. Comparison of the effects of 1.0 Gm. of amphenone and 0.1 Gm. of Tapazole®, each administered one hour prior to the I^{131} tracer test in a hyperthyroid subject.

discharge of all the accumulated radioactive iodine. Thus, the incorporation of radio-iodide into the thyroid hormones or their precursors (so-called "binding") is blocked by amphenone in a manner analogous to that of thiourea and mercaptoimidazole compounds. Similar conclusions have recently been reported in studies on hyperthyroid subjects receiving multiple doses of amphenone (21). The demonstration of this effect of amphenone on organic "binding" does not, however, exclude an additional effect, either primary or secondary, on the thyroidal concentrating mechanism (so-called "trapping").

Amphenone failed to exhibit a discharging effect similar to that of the inorganic anions, perchlorate and thiocyanate (Fig. 5). Tapazole® in a dose of 100 mg. was given by mouth one hour prior to the administration of the I^{131} tracer dose to each of 2 patients with hyperthyroidism. At the fifth hour, 1.0 Gm. of amphenone by mouth failed to induce a discharge of the ac-

cumulated radio-iodide. Although delayed absorption of amphenone was not ruled out in these patients, it appears that amphenone lacks the ability to cause rapid discharge of thyroidal I^{131}. Similar observations have been reported in patients receiving Tapazole® in continuous dosage (17).

Comparison of the inhibitory effect of 1.0 Gm. of amphenone with that of 0.1 Gm. of Tapazole®, each administered by mouth one hour prior to initiation of the I^{131} tracer curve, indicates a similar effect of these goitrogens on the suppression of thyroidal radio-iodide uptake (Fig. 6). The blocking effect of this dose of Tapazole® appears to persist longer than that of the larger dose of amphenone, as shown by the lack of reaccumulation of radio-iodide at twenty-four hours after Tapazole® as contrasted with amphenone.

Thus, in man, amphenone produces inhibition of the organic "binding" of radioactive iodide by the thyroid gland, analogous to that of thiocarbamide or aminobenzene-type goitrogens. It appears to lack any monovalent-anion effect, such as is exhibited by thiocyanate or perchlorate.

In order to obtain therapeutic results in hyperthyroid patients by the administration of amphenone, large doses of this drug must be employed. Because these large doses may produce undesirable side-effects such as drowsiness, psychic depression, gastric irritation and occasionally rash and methemoglobinemia, and because amphenone possesses no striking therapeutic advantage over currently available antithyroid compounds, it appears unlikely that it will merit clinical usage. However, the intriguing nature of this synthetic compound merits further biologic investigation with a view toward elucidating the "common denominator" underlying the biochemical processes of the thyroid and adrenal glands affected by amphenone.

SUMMARY

Amphenone is an unusual chemical compound with diverse biologic properties. In euthyroid and hyperthyroid subjects, a single oral dose of 1.0 Gm. of amphenone will inhibit the thyroidal uptake of radioactive iodine by blocking organic "binding" in a manner analogous to that of the thiourea-type goitrogens. Amphenone does not have a monovalent-anion effect, as does thiocyanate or perchlorate. Multiple doses totaling from 4.0 to 8.0 Gm. completely inhibit thyroidal accumulation of radioactive iodine. It is not anticipated that amphenone will be of clinical value in the therapy of hyperthyroidism. Current interest in this compound may assist in elucidating certain mechanisms which the thyroid and adrenal share in the process of hormonogenesis.

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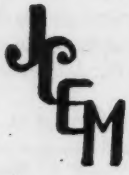

CIRCULATING IODOPROTEINS IN A NONGOITROUS ADULT WITH PRIMARY AMENORRHEA, BONY DEFORMITIES, AND NORMAL LEVELS OF SERUM PRECIPITABLE IODINE AND THYROIDAL I^{131} UPTAKE*

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BLOCK and Werner demonstrated the presence of circulating iodoproteins[1] in the serum of an 8½-year-old nongoitrous cretinous dwarf whose blood was sent by Whitelaw and collaborators (1) for chromatographic analysis. Most of the radioactivity in the serum, seventy-two hour after labelling the thyroid with I^{131}, remained at the origin of the chromatogram with the methods in use at that time (2). Whitelaw and coworkers suggested in their report that the iodoprotein might be thyroglobulin. Recently at our clinic an adult, also without goiter, presented with primary amenorrhea, bony deformities and normal levels of serum precipitable iodine and thyroidal I^{131} uptake; on chromatographic analysis, the serum was found to contain true circulating iodoproteins. The case report and the results of the studies which were conducted are presented here.

METHODS

The methods currently used in our laboratory to concentrate and to identify the iodinated compounds in serum have been described elsewhere (3). By these procedures, it is possible to obtain satisfactory results with a test dose of only 125 μc. of I^{131}. Essentially two methods are employed.

Method I. *Total iodoamino acids and inorganic iodine:* The serum is boiled with hot 1-butanol acidified with sulphuric acid. The filtrate and washings are immediately made

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[1] The terminology used by investigators in the thyroid field is confusing to protein chemists. "Protein-bound" or "serum precipitable" iodine (PBI or SPI) consists of those iodoamino acids which are loosely absorbed to certain serum proteins and which are readily removed from these proteins by denaturation with acids or alkalies. This behavior is distinct from that of the true iodoproteins, such as circulating thyroglobulin, which may also be present in the serum. The iodoamino acids of the true iodoproteins are not removed by such treatment.

alkaline with ammonia. The butanol extract is then concentrated, filtered and diluted with chloroform. The iodinated compounds are extracted from the solvent with normal ammonia. The dried residues (aqueous and solvent phases) are dissolved in methanol ammonia and chromatographed on paper (3).

Method II. *Protein-bound iodine.* The serum, diluted with water, is treated with zinc sulphate and sodium hydroxide (Somogyi reagent). The precipitate is washed, and then suspended in sulphuric acid and boiled to coagulate the proteins. The resulting precipitate is washed with butanol. The butanol extract is made alkaline with concentrated ammonia and the remainder of the procedure is similar to that of Method I.

In the present patient, after circulating iodoproteins were identified, electrophoretic separation of the serum proteins was carried out, using the following method: The proteins in 10 ml. of serum were fractionated on an anion exchange column of 2-chlorotriethylamine-treated cellulose according to the method of Sober *et al.* (4). The fractions obtained from the cellulose columns were identified by paper electrophoresis (5). Absorption at 280 mμ was used to estimate the approximate amount of protein in each fraction. The protein solutions, after concentration *in vacuo* to 3 ml., were counted for radioactivity in a well-type scintillation counter.

The total proteins precipitated in the concentration procedures described were subjected to enzymatic digestion with erepsin and pancreatin. The iodinated compounds from the hydrolysates were concentrated by Method I of Block and collaborators (3) and separated chromatographically with methanol:ammonium acetate.

Serum precipitable iodine levels were determined by the original method of Barker (6). A thyrotropin stimulation test and a triiodothyronine suppression test were conducted by the methods used in our laboratory (7, 8). In the latter test, a 50-μg. daily dose of the hormone is given for eight days instead of the 75-μg. dose originally recommended. The concentration of serum precipitable I^{131} was determined at seventy-two hours by a modification of the method of Chaikoff (9).

CASE REPORT

B. W. (U. H. #266212), a 44-year-old colored woman (Fig. 1) was first admitted on March 13, 1956, because of persistent cough of eight months' duration. She had been born in Georgia and was formerly a cotton picker. A brother had eye lesions and "bad blood." The personal history was irrelevant except for residence in New York City for the preceding eight years, during which time she had not been working. Her height was 5 feet and her average weight was 116 pounds. She had lost 19 pounds in the six months before admission, despite a fair appetite. She had had pneumonia six years before entry, and smallpox two years later. The system review revealed no significant findings except for poor vision since bilateral inflammation of the eyes at the age of 12, and complete failure to mature sexually.

The presenting illness began eight months before entry with a cold and cough. Except for amenorrhea, she had no other complaint. Since onset the cough had been persistent, increasing in severity in the two weeks prior to admission. By the time she entered the hospital, she was producing half a cupful of whitish sputum daily, without blood or pus. She had noticed shortness of breath on walking one block, weight loss, and chronic fatigue. She had neither night sweats nor anorexia.

On physical examination, the patient was obseryed to be a short, slightly hunchedback, mentally dull female with no development of secondary sex characters. There were obvious bilateral corneal scars. The skin was soft and smooth, and the pulse rate was 76. The thyroid was barely palpable but no abnormalities were noted. Except for enlarged

tonsils, a soft systolic murmur at the apex of the heart, and some limitation of motion of the thighs upon abduction, the rest of the physical findings were negative.

Laboratory examination: Hemoglobin level 10.1 Gm. per 100 ml.; red blood cell count 3.4 million; hematocrit 30 per cent; white blood cell count 4,800, with 54 per cent

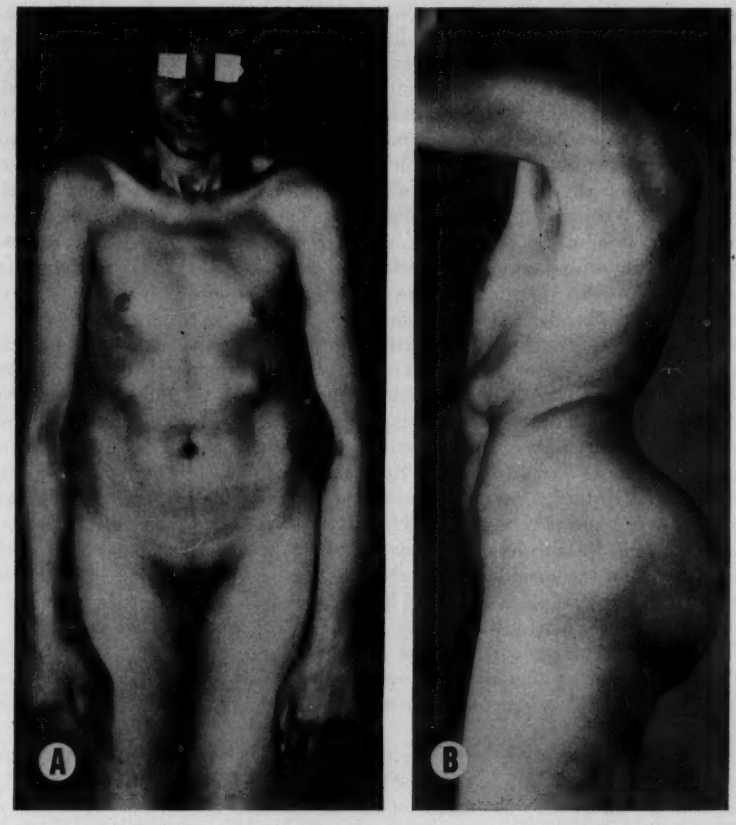

FIG. 1. **A.** Patient B. W., front view showing failure of development of secondary sex characters. **B.** Side view showing lack of breast and axillary hair development, and the round-back deformity of the spine.

polymorphs, 44 per cent lymphocytes and 2 per cent monocytes; Wintrobe red-cell indices—mean corpuscular volume 88 μ^3, mean corpuscular hemoglobin 29.5 $\mu\mu$g., mean corpuscular hemoglobin concentration 34 Gm. per 100 ml.; and erythrocyte sedimentation rate 105 mm. at one hour. Negative findings were obtained with the following tests: sickle-cell preparation, urinalysis, guaiac test for blood in stools, examination of sputum for tubercle bacilli (6 occasions), culture of sputum and gastric washings (2 occasions), inoculation of guinea pigs with sputum for tubercle bacilli, and lupus erythematosus preparation of serum. Results of a tuberculin test were negative at a dilution of 1:10,000

but slightly positive at a dilution of 1:100 (2 cm. erythema). In the histoplasmin skin test and Frei test there was slight erythema at forty-eight and seventy-two hours respectively. The treponema immobilization test and spinal fluid Kolmer test yielded negative findings. Results of the blood Mazzini test were equivocal, and of the Kolmer test, negative.

The concentration of serum albumin was 3.3 Gm. per 100 ml., globulin, 4.7 Gm. per 100 ml., sodium (direct) 138.3 mEq. per liter, potassium 4.5 mEq. per liter, and calcium 11.7 mg. per 100 ml. The 24-hour urinary excretion of 17-ketosteroids was 2.5 mg.; no FSH was detected in the urine. Before infusion of ACTH the plasma corticoid level was 3 μg. per 100 ml.; after infusion it was 9 μg. per 100 ml. The BMR was −41 per cent. The fasting serum cholesterol concentration was 228 mg. per 100 ml. Thyrotropin stimulation test—before TSH, the serum precipitable iodine level was 4.2 μg. per 100 ml. and the 24-hour thyroidal I^{131} uptake was 28 per cent; after TSH the SPI level was 4.6 μg. per 100 ml. and the uptake was 46 per cent. The results of paper chromatographic separation of the iodinated compounds in the serum are presented in the text which follows. Triiodothyronine suppression test—after administration of triiodothyronine for eight days, the 24-hour thyroidal uptake of I^{131} was 4 per cent. Psychometric rating—I.Q. 69–75; "borderline general intelligence."

Roentgenograms of the skull, upper gastro-intestinal tract and small intestines yielded negative results. Roentgenograms of the chest revealed pulmonary fibrosis and emphysema (probably secondary to old tuberculosis), diaphragmatic adhesions, and fibrotic retraction and elevation of the mediastinal structures. This was confirmed in laminograms of the thoracic spine, in which distorted hilar vessels, "probably due to old tuberculous fibrotic lesions" could be seen. Roentgenograms of the thoracic spine showed flattening of the vertebrae, irregularities of the articular plates "probably due to old osteochrondrosis," and a round-back deformity. In roentgenograms of the pelvis, degenerative changes in both femoral heads with flattening and deformity were evident. Roentgenograms of the shoulder girdle, sternum, right wrist and hand showed delayed bone maturation, the roentgen age being 20–25 years at most.

The patient was a diagnostic puzzle, especially in respect to her infantilism and peculiar general body development. The chest lesions were presumed to represent arrested tuberculosis with pulmonary and mediastinal fibrosis, and pulmonary emphysema. The corneal scars were considered to be evidence of childhood keratitis of nonspecific etiology.

Most observers attributed the failure of sexual development to primary hypopituitarism. This view was held to fit best with the evident gonadal failure and with the adrenal failure suggested by the results of the ACTH test. However no etiologic factor could be established to account for damage to the pituitary. Other observers favored secondary hypopituitarism, due perhaps to malnutrition, perhaps to lung disease, or possibly a malabsorption syndrome. Ovarian agenesis was thought to be excluded on the basis of the low titer of urinary gonadotropins and the normal female sex chromatin patterns in scrapings of the oral mucosa. The possibility of hypothyroidism was considered ruled out because of the normal level of serum protein-bound iodine, and the normal response of the thyroidal I^{131} uptake to injection of thyrotropin despite the lack of response of the PBI level. At the end of her hospital stay, one eminent clinician summarized the situation, "just poor protoplasm."

However, the peculiar response to thyrotropin led to further studies, and eventually the patient was shown to be hypothyroid. During thyroid and cortisone therapy, she felt greatly improved.

RESULTS

As indicated under "Methods," the patient's serum was studied by means of paper chromatography, ion exchange chromatography, and electrophoresis. The iodoamino acids in peptide linkage in the proteins remaining after removal of the protein-bound or serum precipitable iodine were further investigated following enzymatic digestion of the proteins.

Iodinated amino acids in serum. The distribution of the iodo-compounds[2] in the serum is shown in Table 1 and Figures 2 and 3. It is seen that 32 per

TABLE 1. DISTRIBUTION OF PROTEINS AND RADIOACTIVITY IN THE PROTEIN FRACTIONS OF SERUM (PATIENT B. W.)

Protein fraction	Approx. distribution of tot. proteins (%)	Radioactivity cts./min./Gm. protein	Distribution of I^{131} (% tot. I^{131} in proteins)
Albumin	41	54	20
Alpha-globulin	16	146	21
Beta-globulin	19	170	29
Gamma-globulin	24	133	29

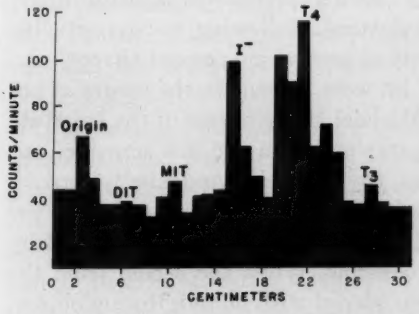

FIG. 2. Distribution of radioactivity in the iodinated compounds of serum; paper chromatogram developed with 2-butanol ammonia (3).

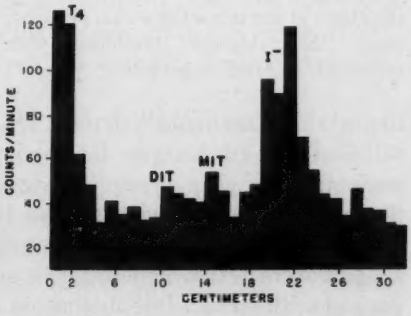

FIG. 3. Distribution of radioactivity in iodinated amino acids of serum; paper chromatogram developed with methanol-ammonium acetate.

cent of the radioactivity, as iodoproteins, was precipitated along with the serum proteins by denaturation with hot acidified 1-butanol. The remainder of the radioactivity was distributed among I^-, T_4, T_3 and MIT.

Distribution of I^{131} in serum proteins. The serum proteins were fraction-

[2] In this paper thyroxine = T_4, triiodothyronine = T_3; diiodothyronine = T_2; diiodotyrosine = DIT; monoiodotyrosine = MIT; and inorganic iodide = I^-.

ated by ion exchange chromatography (4, 10). The proteins and the radioactivity contained in them after labelling with I^{131} were distributed as shown in Table 1.

Serum from a healthy control subject analyzed concurrently showed the following protein distribution pattern: albumin 56 per cent, alpha globulins 15 per cent, beta globulins 12 per cent, and gamma globulins 16 per cent.

Iodinated compounds in circulating iodoprotein. The distribution of I^{131} obtained on a methanol:ammonium acetate chromatogram after enzymatic hydrolysis of the serum protein precipitate remaining after removal of the protein-bound iodine by the concentration procedure listed under "Methods," is shown in Figure 4. The I^{131} in the protein precipitate was distributed among T_4 and T_3, DIT, and I^-. MIT, if present, was too small in amount to be detectable.

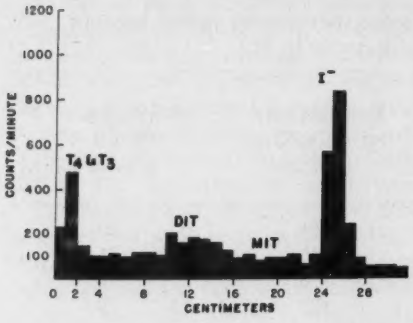

FIG. 4. Distribution of radioactivity in the digest of the extracted serum proteins; paper chromatogram developed with methanol: ammonium acetate.

Serum precipitins against thyroglobulin. Following the report (16) that precipitins against thyroglobulin were present in the serum of patients with Hashimoto's struma, Dr. Michael Heidelberger of the Institute of Microbiology, Rutgers University, was asked to test the serum of our patient, B. W., for precipitins against thyroglogulin and kindly agreed. Sera from a patient with typical Hashimoto's struma (according to the pathologic report on tissue removed at operation) and from a healthy subject were tested concurrently as controls. When the serum from the patient with struma lymphomatosa was placed with human thyroglobulin, a precipitate developed; the concentration of antibody antigen was approximately 5 μg. per ml. of serum. In the serum of the control subject and of Patient B. W. there was no visible precipitin response by as late as two weeks.

DISCUSSION

It is clear that our patient's thyroid was elaborating iodoproteins which did not yield their amino acids when boiled with acid butanol. Approximately one third of the serum radioactivity was in this form. Fractionation of the serum proteins by the method of ion exchange chromatography showed that the radioactivity was not concentrated only in the alpha-globulin and albumin fractions, as was to be expected from the analyses

of normal sera by Larson and coworkers (11) and by Gordon and collaborators (12). Radioactivity also was present in significant amounts in the beta and gamma globulins. Binding of T_4 by the gamma globulins has been reported only once, by Robbins and associates in a single patient with a carcinoma of the thyroid (13).

The serum protein-bound radioiodine was present largely as T_4, and I^-, with some T_3 and MIT but no detectable DIT. The circulating iodoproteins on the contrary appeared to have more DIT than MIT; however, fractionation of the total serum proteins was undertaken and hence the results are complicated by the presence simultaneously of both the serum proteins and the circulating iodoproteins. Stanley found the ratio MIT: DIT to be 1:1 in the tryptic hydrolysate of the proteins of normal human thyroid tissue, essentially thyroglobulin. This suggests that the iodoproteins of our patient may have been different from, or were an altered, thyroglobulin. The ratio of MIT to DIT in thyroglobulin can vary, since Stanley found it to be 5:1 in the protein hydrolysate from malignant thyroid tissue (14).

In this connection, also, it is of interest that Owen and McConahey (15) in patients with the Hashimoto form of chronic thyroiditis found circulating iodoproteins which they suggested might represent thyroglobulin. Their view is bolstered by the recent demonstration by Roitt and coworkers (16) that precipitins against thyroglobulin are present in the serum of such patients, and the confirmation in the patient with the disorder tested in our own study.

It is thus possible that our patient, B. W., had long-standing chronic thyroiditis and that her gland became atrophic and hence impalpable on physical examination. However, autoimmunization to thyroglobulin does not seem to have been the cause of thyroiditis in her case since there were no precipitins of this nature demonstrable in her serum. Moreover, the iodoproteins in her serum were not typical of thyroglobulin when compared with the data of Stanley, although the latter are limited. Finally, her past history did not suggest that she had ever had enlargement of her thyroid. Thus the likelihood that thyroiditis accounted for the presence of iodoproteins in the serum is poor. An alternative explanation for leakage of iodoproteins could be the presence of a defect in proteolysis of the colloid. Less likely, perhaps, is the possibility of synthesis by the gland of an abnormal protein which then leaked from the gland. Both these last alternatives fail to explain the absence of goiter.

Hypothyroidism presumably accounted for the primary amenorrhea, defective bony development and poor adrenocortical response to ACTH in the present patient, and also for stunting in the cretin reported by Whitelaw *et al.* (1). Normally, thyroxine is readily released from the carrier

proteins. When circulating iodoproteins contain the bulk of the thyroxine, this fraction of the hormone cannot be released to the tissues until the protein is metabolized. Thus in Patient B. W., about a third of the circulating thyroxine, as judged by the radioactivity, was rendered poorly available, if at all. Only a third of the serum precipitable iodine was thyroxine normally bound to carrier proteins (Table 1). With an SPI level of 4.2 μg. per 100 ml., the amount of hormone reaching the tissues would be in the hypothyroid range.

The syndrome presented by Patient B. W. may exist on a wider scale than is presently evident. The failure of the serum precipitable iodine and thyroidal I^{131} uptake determinations to reveal her hypothyroid status suggests that such failure may have obscured the underlying pathologic mechanism in other patients with amenorrhea or related disorders. Thus, the wider use of chromatographic procedures may be indicated. When this technic is not available, two alternative methods can be used. One is the injection of thyrotropin to stimulate the thyroid. The other is the determination of the levels of both serum precipitable and butyl extractable iodine (17). Failure to respond to thyrotropin, or a discrepancy between the values from the latter two determinations, would suggest the possibility that circulating iodoproteins were present, and the need for chromatography.

SUMMARY

1. Circulating iodoproteins have been found in a nongoitrous cretinoid adult with primary amenorrhea, bony deformities, and normal levels of serum precipitable iodine and thyroidal I^{131} uptake.

2. A study of the patient's serum was made by paper chromatography, electrophoresis, and by hydrolysis and subsequent chromatography of the serum proteins. Approximately a third of the protein-bound iodine consisted of circulating iodoproteins, and a third consisted of thyroxine bound to alpha globulins and albumin. Twenty-nine per cent of the circulating radioactivity was bound to the gamma globulins and a similar amount to the beta globulins.

3. Precipitins against thyroglobulin were not demonstrable in the serum of this patient or in that of a control subject, although present in the serum concurrently tested of a patient with proven Hashimoto's struma, in confirmation of the finding of Roitt and co-workers.

4. Since the circulating iodoproteins had to be metabolized before they could release thyroxine to the tissues, and since only a third of the serum precipitable iodine was thyroxine normally bound to carrier proteins, it is postulated that the patient was hypothyroid despite the normal level of serum precipitable iodine. The patient's chemical defect may have resulted from long-standing chronic thyroiditis; alternatively, defective

proteolysis, or synthesis of an abnormal protein, may have permitted the leakage of iodoproteins into the circulation.

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PLASMA CORTICOSTERONE AND HYDROCORTISONE LEVELS IN MAN

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CORTICOSTERONE and hydrocortisone have been demonstrated to be present in human adrenal vein blood (1) and adrenal gland perfusates (2). Selective methods for quantitation of plasma hydrocortisone levels in peripheral blood have been described (3, 4) and their specificity demonstrated (5, 6). Methods have been proposed for the determination of "corticosterone-like" material in the plasma; however, data have not been available on either the specificity or quantitative validity (accuracy and precision) of these procedures.

Recently, a specific method has been described for the assay of corticosterone in the peripheral blood of man (7). This method utilizes the principle of isotope dilution to correct for losses of steroid during the extraction and chromatography processes, and the purified sample of corticosterone is assayed by a modification of the fluorescence method of Sweat (8). With this method, it is possible to detect 0.2 μg. of corticosterone per 100 ml. of plasma.

In view of the fact that no reports have previously been published comparing plasma corticosterone and hydrocortisone levels, it was deemed of interest to report such data in patients with various adrenal disorders, and in normal subjects before and after experimentally altered states of adrenocortical activity.

METHODS

In 17 patients with various endocrine disorders and in 30 normal subjects, the plasma corticosterone level was determined by the isotope dilution procedure as previously described (7), and the hydrocortisone level by a modification of the Silber-Porter method (3) as previously described (5). This method has been shown to be highly specific for plasma hydrocrotisone with but few exceptions. The normal subjects used in this study consisted of 18 men and 12 women between the ages of 17 and 40 years. Corticotropin (ACTH) was continuously infused at the rate of 4 units per hour for eight to seventy-two hours.

RESULTS

Table 1 lists the levels of corticosterone and hydrocortisone in the plasmas of the normal subjects and of the patients with various alterations in adrenal function. Patient M.F. with Addison's disease had significantly

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TABLE 1. PLASMA CORTICOSTERONE AND HYDROCORTISONE LEVELS IN NORMAL SUBJECTS AND IN ADRENAL DISEASE STATES

	Corticosterone (μg./100 ml.)	Hydrocortisone (μg./100 ml.)
Normal Subjects	0.4–2.0	6.0–25.0
18 male; 12 female	1.1 ±0.4	14.0 ±1.2
Addison's Disease		
C.G.	0.2	1.0
M.F.	0.6	3.0
G.B.	0.3	0
S.F.	0	0
Hypopituitarism		
R.P.	0	3.0
A.K.	0.2	0
Adrenogenital Syndrome		
B.O.	0.6	1.0
S.S.	0.4	4.0
O.S.	0.4	4.0
Cushing's Syndrome		
Unilateral tumor		
V.N.	1.4	33.0
R.E.	1.1	23.0
Bilateral hyperplasia		
V.W.	1.0	60.0
A.L.	0.7	38.0
Carcinoma		
N.G.	1.5	70.0
V.G.	2.0	156.0
G.T.	1.7	67.0
G.V.	0.7	23.0

measurable quantities of corticosterone and hydrocortisone in the plasma; however, there was no increase in the concentration of these steroids after a 48-hour infusion of ACTH (Table 2). In the patients with Cushing's syndrome, the plasma corticosterone levels were within the normal range, whereas with 1 exception (R.E.), the plasma hydrocortisone levels were significantly elevated. In 1 patient (V.G.), the plasma corticosterone level was elevated to 5.6 μg. per 100 ml. (Table 2) following discontinuance of amphenone therapy and a few weeks prior to death. With the exception

TABLE 2. PLASMA CORTICOSTERONE AND HYDROCORTISONE LEVELS FOLLOWING EXPERIMENTALLY INDUCED ALTERATIONS IN ADRENAL FUNCTION

	Corticosterone (μg./100 ml.)	Hydrocortisone (μg./100 ml.)
Normal Subjects:		
After 8 hrs. intravenous ACTH		
J.B.	15.0	46.0
T.R.	8.0	40.0
N.K.	10.0	50.0
J.E.	11.0	37.0
M.P.	7.0	40.0
Ten hours after 2 mg. Δ^1,9α-fluorohydrocortisone orally		
T.R.	0	1.5
J.E.	0	0
Before intravenous Piromen	1.8	15.0
Two hours after intravenous Piromen*	3.0	25.0
Before surgery	0.5	14.0
Four hours after surgery	3.0	36.0
Before amphenone	1.1	20.0
Amphenone, orally, 5 Gm. q. day ×5	0.4	9.0
Addison's Disease (M.F.):		
Control	0.6	3.0
Forty-eight hours after intravenous ACTH	0.4	3.0
Carcinoma of Adrenal (V.G.):		
Control	2.0	156.0
Amphenone, intravenously, 1 Gm. q. hr. ×8	0.5	38.0
After amphenone	5.6	240.0

* Dose = 0.2 μg. per Kg. body weight.

of the group of patients with Cushing's syndrome, an increased level of corticosterone was always associated with an increased level of hydrocortisone, and also a decreased concentration of corticosterone was associated with a decreased concentration of hydrocortisone (Table 2).

Figure 1 depicts the increased levels of hydrocortisone and corticosterone in the plasma following continuous infusion of ACTH for seventy-two hours in 2 normal subjects. Six to twelve hours after the start of ACTH stimulation, there was a decreased ratio (3:1 and 5:1) of hydrocortisone to corticosterone over the control value (13:1); however, this lowered ratio

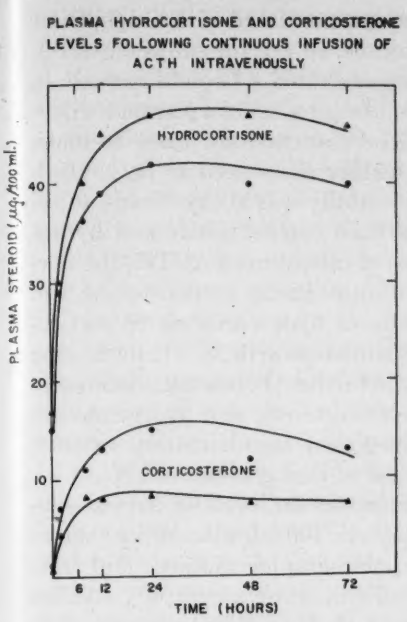

FIG. 1. Plasma hydrocortisone and corticosterone levels following continuous infusion of ACTH intravenously.

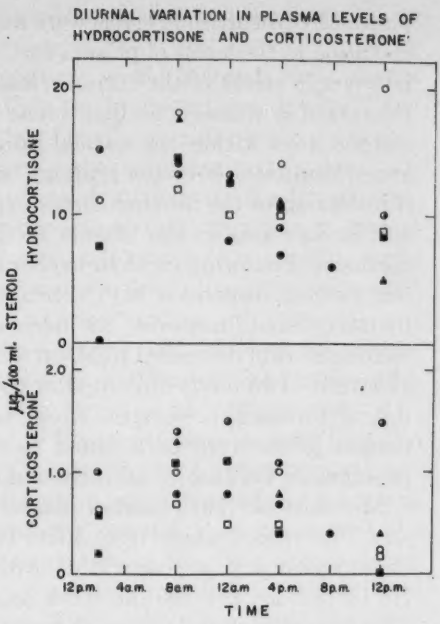

FIG. 2. Diurnal variation in plasma levels of hydrocortisone and corticosterone.

remained essentially constant over the period from twelve to seventy-two hours.

Figure 2 shows the close correlation between the diurnal variation of plasma levels of both hydrocortisone and corticosterone in 6 normal subjects.

DISCUSSION

The plasma hydrocortisone-to-corticosterone ratio in normal subjects was found to average about 13:1. Patients with adrenal disorders associated with a decreased production of hydrocortisone, such as Addison's disease, hypopituitarism and the adrenogenital syndrome, were found to have low or unmeasurable levels of plasma corticosterone. In 2 patients with Addison's disease, intravenous ACTH failed to elevate the levels of either corticosterone or hydrocortisone. Experimental alterations in adrenal function through suppression of the pituitary with Δ^1,9α-fluorohydrocortisone, or direct suppression of the adrenal with amphenone, produced a decrease in the levels of both corticosterone and hydrocortisone.

In the hyperadrenal states studied (Cushing's syndrome), the increased

plasma hydrocortisone levels were not always associated with a significant elevation in the levels of plasma corticosterone. In 1 of the subjects (G.T.), however, a study of the turnover rate demonstrated a twofold increase in the rate of synthesis of corticosterone (9) in the presence of a plasma corticosterone level within the normal range. There are too few cases to make generalizations about the findings, and further cases need to be studied. Stimulation of the adrenal cortex experimentally was always found to result in increases in the plasma levels of both corticosterone and hydrocortisone. Following eight to twelve hours of intravenous ACTH, the normal subject responded with increases in both plasma corticosterone and hydrocortisone; however, the normal ratio of hydrocortisone to corticosterone of 13:1 decreased to about 4:1. Stimulation with ACTH for as long as seventy-two hours did not alter the ratio further. Following administration of Piromen, or surgical stress, the corticosterone and hydrocortisone plasma levels were both found to be increased approximately twofold, presumably because of an increased release of endogenous ACTH.

Morris *et al.* (10) reported plasma corticosterone levels in normal subjecs. The values ranged from 4.0 to 10.5 μg. per 100 ml. when using column chromatography in conjunction with a polarographic method, and from 7.8 to 15.8 μg. per 100 ml. when using column chromatography, alkaline blue-tetrazolium and ultraviolet assay methods (11). Weichselbaum *et al.* (12) utilized column chromatographic purification and alkaline blue-tetrazolium colorimetric assay, and reported the mean plasma level of corticosterone-like ("17-desoxycorticosteroid") material to be 8.9 ± 2.0 μg. per 100 ml. Sweat (1), using column chromatography and a sulfuric acid-ethanol reagent for determination of fluorescence, reported a mean concentration of corticosterone-like material of 4.3 ± 2.3 μg. per 100 ml. Bush (13), from paper chromatograms of normal plasma extracts, estimated the corticosterone concentration to be 2 μg. per 100 ml. or less.

Some discussion of the results reported in this paper and the work of Bush, as contrasted with the reports of Morris, Weichselbaum, and Sweat, would appear to be in order. Studies of the eluates from the paper chromatograms of the plasma extracts, in the region slightly more distal and more proximal to corticosterone, show that substances other than corticosterone are present in plasma that give rise to a variable but significant degree of measurable ultraviolet absorption at 240 mμ, alkaline blue-tetrazolium reduction, and sulfuric-acid fluorescence. Thus, it is conceivable that with the less precise column chromatographic separations, a variable amount of this material may be included in the corticosterone fraction. Also, it is possible that these same substances that absorb in the ultraviolet region at 240 mμ and are blue-tetrazolium reactive might interfere with the polarographic methods. The fact that added corticosterone was recovered

satisfactorily from plasma does not prove that the method was selective for corticosterone.

In addition, the following observations would suggest that plasma corticosterone levels are much lower than the hydrocortisone levels. There is abundant evidence to show that the phenylhydrazine-reactive tetrahydro steroid derivatives of hydrocortisone make up approximately one quarter of the urinary steroid metabolites of oral or intravenously administered, as well as endogenously produced, hydrocortisone (14). However, in normal urine there are only traces of the tetrahydro derivatives of corticosterone or 11-dehydrocorticosterone (15), whereas these derivatives may be present in abundant quantity in the urine following oral or intravenously administered corticosterone (15–17). This suggests that, like hydrocortisone, the tetrahydro metabolites are formed from corticosterone. Isotope dilution assays of urine have shown the daily excretion of corticosterone to be about 2 to 6 μg. (15, 18), whereas that of hydrocortisone has been found to be in the range of 30 to 90 μg. (ratio 1:15) (18–20). Corticosterone turnover-rate studies (21) have shown that the miscible pool of corticosterone is about 200 μg., and this is approximately one eighth of the size of the hydrocortisone pool. The pool of corticosterone has been found to be turned over approximately seven times per day, resulting in a total daily corticosterone production of 2 to 3 mg. This is about one tenth the daily production of hydrocortisone (22). These turnover data suggest that corticosterone is metabolized at a rate not greatly different from that of hydrocortisone; therefore, the low urinary values for corticosterone cannot be explained on the basis of a slower turnover of a larger pool. These low urinary values may result in part from a low renal clearance of corticosterone caused by the low concentration of nonprotein-bound steroid available for filtration (15, 23). Following the infusion of corticosterone, it has been found that the steroid disappears at a rate only slightly more rapid than that of hydrocortisone (15)—the half-time being seventy to ninety minutes, as contrasted with an average half-time of one hundred and ten minutes for hydrocortisone.

Thus, the average plasma hydrocortisone-to-corticosterone ratio in normal subjects is about 13:1, the hydrocortisone-to-corticosterone concentration in the urine about 15:1, the miscible pool of hydrocortisone to that of corticosterone 8:1, and the quantity (mg.) of each synthesized per day about 10:1—in the presence of a rate of metabolism of corticosterone that is only slightly greater than that of hydrocortisone.

There was a diurnal variation in the plasma corticosterone levels in normal subjects which was correlated with the diurnal variation in plasma hydrocortisone levels. During the diurnal change, the ratio of hydrocortisone to corticosterone remained essentially unchanged. The diurnal

variation in hydrocortisone was of a similar pattern to that reported previously (24).

SUMMARY

Comparative values for plasma corticosterone and hydrocortisone levels in normal subjects and in patients with various natural or experimentally induced adrenocortical disorders have been presented. The average ratio of hydrocortisone to corticosterone in normal subjects was found to be 13:1. Natural and experimentally induced alterations in adrenocortical function (except in the case of Cushing's disease) were associated with changes in both plasma corticosterone and hydrocortisone levels. Intravenous corticotropin administered continuously for eight to seventy-two hours resulted in a decrease of the plasma hydrocortisone-to-corticosterone ratio to about 4:1. A diurnal variation in the level of plasma corticosterone was found to parallel the diurnal variation in the level of hydrocortisone.

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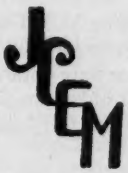

STUDY OF POTENTIALLY ACTIVE ADRENOCORTICAL STEROIDS IN PERIPHERAL HUMAN PLASMA AFTER VARIOUS STRESSES*

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THE present observations deal with chemical measurements of potentially biologically active adrenocortical steroids, *i.e.*, those containing a Δ^4-3-keto grouping, together with corticosteroids measured by previously described methods. It is believed that these new procedures furnish a more accurate means of measuring adrenocortical activity and thus have greater diagnostic possibilities than methods now in use, particularly in states of clinical adrenocortical insufficiency or failure. The data also add considerable information regarding probable metabolic changes that various steroids may undergo.

METHODS

In a previous communication (1), free and conjugated Porter-Silber (PS) reactive materials (17-hydroxycorticosteroids) and the differences between them and free blue-tetrazolium (BT) reactive materials (17-desoxycorticosteroids) were measured in the peripheral plasma of patients undergoing elective surgical procedures of various kinds. In the present communication, free and conjugated Δ^4-3-ketocorticosteroids were measured by a new technic, previously described (2), involving the use of isonicotinic acid hydrazide and referred to here as the INH reaction. The special significance of this reaction is due to the probability that only potentially biologically active steroids are measured thereby. Also included are determinations of glucuronic-acid conjugates of BT-reactive materials not previously reported.

Four groups of patients were studied as follows:

I. *Infusion of corticotropin (ACTH).* Two "normal" patients (Cases #1 and #2) in stable clinical condition and without obvious abnormality were used as subjects. An intravenous infusion of 15 mg. of ACTH was given during four hours on the day preceding the actual test in order to "activate" the pituitary gland—a procedure which has been demonstrated as important in elucidating ACTH activity by previous observers (3). On the day of the experiment, an intravenous infusion of 40 mg. of ACTH was given during eight hours. Preliminary blood samples were obtained before the "activating" dose and before the actual infusion. Subsequent blood samples were taken at the middle and end of the study—at sixteen, twenty-four and forty-eight hours after the infusion.

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II. *Gastrectomy.* From 2 male patients blood samples were obtained before gastrectomy, eight and ten hours after the incision was made, and twenty-four and forty-eight hours later.

One of the patients (Case #3) was a 66-year-old man who had had epigastric pain and distress for several months before admission and in whom, after a barium meal, there was radiographic evidence of a gastric ulcer. Despite many weeks of hospitalization and intensive medical therapy, the radiologic evidence persisted and he was subjected to a gastric resection. The ulcer was found and removed as part of a gastric resection which took two and a half hours to complete. Following this operation he had an uneventful convalescence and left the hospital in two weeks. His preoperative and postoperative laboratory data showed little deviation from normal.

The other patient (Case #4) was a 52-year-old man with a three-month history of dyspepsia and pain relieved by taking food. Several hours before admission he vomited a large amount of blood, following which he fainted. Later his stools were tarry. On admission his hemoglobin level was only 7 grams per 100 ml., but he was not in a state of shock. After transfusions of 1500 ml. of whole blood, the hemoglobin level rose to 10 grams per 100 ml. He was operated upon two days later. A duodenal ulcer was found, for which a gastric resection was performed. The procedure was difficult and required six and a half hours. The postoperative course was somewhat stormy, but eventually recovery was complete.

III. *Severe burns.* The 2 patients studied were young captains (co-pilots) in the U. S. Air Force, burned on September 7, 1955, when a B-25 photoship was hit by an FO86 aircraft. The accident followed an attempted landing of the B-25 at McCarran Field, Las Vegas, Nevada. The aircraft was demolished by fire and all crew members severely burned. Both patients were admitted to the 3595th U.S.A.F. Hospital, Nellis AFB, Nevada, about thirty minutes after the crash. Neither patient was in pain and both were conscious and rational.

Case #5. In this patient, soon after admission, profound shock and severe respiratory distress developed. The latter was relieved by an immediate tracheotomy. A "cutdown" was made in a vein in the left ankle for continuous venoclysis, antibiotics were started, and the patient was transferred to the Surgical Research Unit at the Brooke Army Hospital. Examinations there revealed a critically ill, unconscious patient with burns which covered almost the total (90 to 95 per cent) area of his body; the great majority were undoubtedly of the deep third-degree type. The urine was grossly bloody and amounted in all to only 495 ml. Because of his critical condition, hypothermia was induced and reached 85° F., but was discontinued when evidence of AV block appeared in the electrocardiogram. Laboratory data showed a hemoglobin level of 18.5 Gm. per 100 ml. dropping to 10.5 Gm., white cell count 23,100 dropping to 14,540, plasma sodium level (constant) 142 mEq./L., potassium 5.6 mEq., chlorides 115 mEq., and CO_2 18 and 23 mEq./L. A total of 9,225 ml. of fluids was given during the fifty hours the patient lived.

Case #6 was similar to Case #5, but the patient lived eight days. During this period he received 5 liters of whole blood, 4 liters of plasma, and 2 liters of dextran and other fluids. Urinary output was adequate; the urine was bloody at first, but cleared later. Evidence of pulmonary edema appeared but was controlled by reducing the flow of intravenous fluid. Emesis, present at the outset, stopped and oral fluids were retained on the sixth day. Laboratory study showed an initial hemoglobin level of 20.6 Gm. per 100 ml., which dropped and remained at 11 to 13 grams per 100 ml. Plasma electrolyte

concentrations were within normal range except for a slight persistent elevation of the potassium level (5–6 mEq./L.), a terminal fall in the sodium level to 122 mEq./L., and a fall in the CO_2 level to 17 mEq./L.

The concentration of nonprotein nitrogen increased gradually up to 150 mg. per 100 ml.

IV. *Terminal cancer.* Several patients with terminal breast cancer were being cared for in the Homer G. Phillips Hospital until they expired. Peripheral blood samples were obtained for analysis at intervals of one month or more.

The analytical methods used have been previously described (2, 4).

RESULTS

The findings are presented in Tables 1–4.

For purposes of simplicity, Porter-Silber reactive materials (17-hydroxycorticosteroids) will be designated as PSR, free, conjugated and total; materials reactive to isonicotinic acid hydrazide (Δ^4-3-ketocorticosteroids), as INHR, free, conjugated and total; and alkaline blue-tetrazolium reducing materials (alpha-ketol steroids), as BTR, free, conjugated and total. For convenience in interpreting BTR values, the PSR values calculated as hydrocortisone can be subtracted from the BTR values also calculated as hydrocortisone, in order to obtain a numerical value for certain "alpha-

TABLE 1. LEVELS OF ADRENOCORTICAL STEROIDS (μG./100 ML.) IN PERIPHERAL PLASMA OF 2 "NORMAL" SUBJECTS FOLLOWING INFUSION OF ACTH

Hours	PS*-reactive			INH*-reactive			BT*-reactive		
	Free	Conj.	Total	Free	Conj.	Total	Free	Conj.	Total
Case #1									
−24	16	20	36	6	18	24	30	81	111
0	25	17	42	14	22	36	40	75	115
4	62	40	102	47	26	73	77	97	174
8	87	83	170	86	<5	88	117	161	278
12	90	30	120	40	44	84	90	148	238
24	24	26	50	21	16	37	39	54	93
Case #2									
−24	14	16	30	10	14	24	21	81	102
0	11	12	23	9	<5	12	11	57	68
4	40	41	81	33	14	47	51	116	167
8	41	56	97	39	53	92	47	150	197
12	30	48	78	25	<5	27	38	144	182
24	13	22	35	8	13	21	17	68	85

* In this and subsequent tables:
PS = Porter-Silber.
INH = Isonicotinic acid hydrazide.
BT = Blue tetrazolium.

TABLE 2. LEVELS OF ADRENOCORTICAL STEROIDS (μG./100 ML.) IN PERIPHERAL PLASMA FOLLOWING GASTRECTOMY

Hours	PS*-reactive			INH*-reactive			BT*-reactive		
	Free	Conj.	Total	Free	Conj.	Total	Free	Conj.	Total
Case #3									
0	<5	22	27	<5	22	26	14	86	100
8	30	44	74	55	<5	58	30	119	149
24	27	28	55	<5	52	56	27	122	149
48	15	39	54	10	31	41	22	102	124
72	8	26	34	20	8	28	17	83	100
336	16	14	30	18	6	24	32	43	75
Case #4									
0	21	15	36	24	<5	27	31	34	65
8	81	32	103	76	<5	78	81	102	183
24	68	69	137	54	20	74	79	119	198
48	36	27	63	27	23	50	42	52	94
120	16	9	25	18	8	26	25	19	44

* See footnote to Table 1.

ketol steroids" that are not Porter-Silber reactive. All results are listed in terms of micrograms per 100 ml. of plasma expressed as hydrocortisone equivalents. In many instances a concentration of less than 5 micrograms per 100 ml. is recorded, because the limitations of the analytical method did not permit accurate determination of such small amounts.

TABLE 3. LEVELS OF ADRENOCORTICAL STEROIDS (μG./100 ML.) IN PERIPHERAL PLASMA OF PATIENTS WITH SEVERE FATAL BURNS

Days after burn	PS*-reactive			INH*-reactive			BT*-reactive		
	Free	Conj.	Total	Free	Conj.	Total	Free	Conj.	Total
Case #5									
1	63	188	251	46	17	63	123	—	—
Case #6									
0	43	189	232	9	58	67	61	—	—
2	48	205	253	11	46	57	102	—	—
3	41	220	261	36	35	71	132	—	—
4	42	197	239	19	30	49	119	—	—
5	64	207	271	46	11	57	207	—	—
7	54	186	240	42	45	87	128	—	—

* See footnote to Table 1.

TABLE 4. LEVELS OF ADRENOCORTICAL STEROIDS (μG./100 ML.) IN PERIPHERAL PLASMA OF PATIENTS WITH TERMINAL MAMMARY CANCER

Patient	Months before death	PS*-reactive			INH*-reactive			BT*-reactive		
		Free	Conj.	Total	Free	Conj.	Total	Free	Conj.	Total
1. H.H.	3	21	8	29	<5	34	36	21	109	130
	½	15	21	36	<5	<5	<5	56	96	152
2. E.P.	3	20	<5	25	<5	13	15	34	73	107
3. A.B.	1	6	33	39	<5	21	24	14	116	130
4. I.D.	8	21	18	39	<5	18	20	21	51	72
	6	33	15	48	50	6	56	73	68	141
	3	<5	36	38	7	13	20	9	85	94
	2	26	26	52	50	<5	50	52	68	120
5. H.B.	¼	16	24	40	45	20	65	35	66	101
6. C.J.	1	13	17	30	14	<5	16	44	117	161
7. W.T.	½	62	16	78	36	11	47	87	27	114
8. T.W.	1	22	6	28	23	<5	26	40	82	122

* See footnote to Table 1.

As shown in Tables 1 and 2, following either the four-hour infusion of ACTH or gastrectomy the steroid patterns were similar, except that the blood levels remained high for a longer time following gastrectomy. In both instances there was little or no difference between the free PSR and the free BTR values, indicating a virtual absence of any "alpha-ketol corticosteroids" which are not Porter-Silber reactive. This confirms earlier findings noted after other operations (1).

In nearly all categories (Tables 1–4) the free PSR values were substantially higher than the free INHR values. Special attention is drawn to Case #1 (Table 1) at twelve hours. The variations in the pattern of the PSR and INHR conjugates as shown in Tables 1, 2 and 3 are noteworthy, in that the rises and falls were frequently reciprocal. These findings appear to have definite bearing on the near-zero values found in Case #3 (Table 2) for free INHR materials.

In most of our observations the plasma levels of free BTR materials were higher than the corresponding levels of free INHR materials, but there were 2 notable exceptions (both in Case #3, Table 2) in which the values for free INHR were higher than those for free BTR. The free BTR materials include (in micrograms per 100 ml. equivalents) the unconjugated steroids, which are Porter-Silber reactive. These exceptions have important metabolic significance, which will be discussed subsequently.

A comparison of the 2 cases presented in Table 2 shows marked differences in free PSR and free INHR values. However, when comparing total

PSR and the total INHR values these differences are much less apparent.

In the 2 burn cases (Table 3) there were elevated levels throughout. The levels of PSR conjugates were noteworthy, being as high as 220 micrograms per 100 ml, or two to five times greater than the highest level found after a four-hour infusion of ACTH or a gastrectomy. Worthy of comment also were the consistently higher free PSR values as compared to free INHR values.

In terminal cancer cases (Table 4) the pattern of steroids was variable. The fluctuations from month to month were fairly wide and could not, moreover, be correlated with changes in the clinical behavior of the patients. These findings are presented, not as examples of the pattern in terminal cancer, but rather as the pattern in a group of patients who are severely ill, in order to show the marked variations from values found in "normal" subjects (2). The absence of any measurable quantity of free INHR material in 6 out of 10 samples of peripheral plasma is especially noteworthy.

COMMENT

The data presented have many applications regarding the adrenocortical response to certain stimuli, not only in terms of the increased secretion of corticosteroids, but also of the variations in their peripheral metabolic fate.

The limitations in the methods used must be emphasized. Absolute specificity cannot be expected from chemical reactions involving such a relatively crude extract as is obtained by organic solvent extraction of plasma and Florisil chromatography. The Porter-Silber reaction has been extensively used under such conditions and at present is generally believed to be fairly specific—more so certainly than the alkaline blue-tetrazolium reduction method. However, neither reaction furnishes information as to the quantity of potentially biologically active steroids. The latter, we believe, are estimated by the INH reaction, and the relative specificity of this procedure has been previously discussed (2).

In the present study we have been mainly concerned with the INHR material. This reaction measures only steroids possessing a Δ^4-3-keto grouping, which is significant physiologically because it is known to be present in all steroids possessing biologic activity. The data reported here, therefore, provide information obtained by chemical means of the probable amount of potentially biologically active steroids in the peripheral blood of the human subject after various stresses. It will be noted that the free INHR values deviate in both directions from the free PSR values. Confirmation of these findings may be found in the report by Migeon and associates (5), who compared PSR values with those for hydrocortisone as de-

termined by paper chromatography and found similar but less pronounced differences.

The values for free INHR material recorded here were in general smaller than those for free PSR material, especially in the face of severe stress (Table 3), indicating that free PSR levels do not necessarily furnish an accurate estimation of the amount of potentially biologically active steroids. That the Porter-Silber reaction measures inactive steroids, such as free tetrahydro E and F, is well known. However, it has been generally assumed that these steroids are not present in human peripheral blood in the free form, because none of the isolation studies on unhydrolyzed human blood (6, 7) has indicated the presence of these compounds. We believe the reason for the lack of detection of free tetrahydro E and F is the fact that these studies were made on blood samples from human subjects or animals that were not under stress. With the fairly gross paper chromatographic technics employed, relatively small normal amounts of tetrahydro E and/or F would likely not be detected. Under certain conditions, tetrahydro E and/or F may be present in large amounts and indeed account in some blood samples (Table 3) for up to 50 per cent or more of PSR material. That the presence of these compounds could be due to a spontaneous hydrolysis of tetrahydro E and F glucuronic-acid conjugates during storage in the frozen state has been investigated, but no change was found in the level of free PSR material in frozen plasma samples stored as long as three months. The fact that the proportion of free inactive steroids tends to be much greater the more serious the clinical state of the patient, is of interest and of possible physiologic significance. By contrast there is little or no difference between the PSR and INHR values in the preliminary ACTH infusion samples and in the pre- and post-gastrectomy samples, as shown in Tables 1 and 2. This is in agreement with "normal" values previously reported (2).

The explanation of the 2 determinations (Table 2, Case #3) in which the free INHR values were higher than the free PSR or *even* the free BTR values may be the presence of certain 20-hydroxy compounds, such as Reichstein's compound E, which contain the Δ^4-3-keto grouping. Such compounds would produce an INH but *not* a Porter-Silber or alkaline BT reaction. In a recent report, Burstein (8) pointed out that such highly hydrophilic compounds would be present in the ethyl-acetate extracts which we have used (4) in much greater proportion than in halogenated hydrocarbon extracts used by other workers (9, 10). Although these 20-hydroxy compounds are biologically inactive, they have been isolated from the adrenal cortex (11) and have been produced by *in vitro* incubation with liver slices (12), and certain of their metabolites have been isolated from urine (13).

The increase in the plasma PSR level reported here as evidence of

adrenocortical stimulation is fairly conclusive and confirmatory of previous findings by others and by ourselves. Confirmatory, too, is the failure of alpha-ketol steroids not yielding a Porter-Silber reaction to share in the rise of PSR values, either after administration of ACTH or after operation. This suggests either a differential adrenocortical response to the stimulus as regards the two groups of steroids, or a difference in their relative metabolism in the peripheral tissues.

The absence of free INHR material in certain blood specimens after gastric resection is an interesting observation. Considered only from the functional point of view, it would seem to indicate an apparent state of adrenocortical insufficiency, even though the patients exhibiting this phenomenon demonstrated no detectable clinical evidence of that disorder. However, in the patient (Case #3) in whom free INHR values were virtually zero, the INHR conjugates were distinctly elevated above average normal values (2). These conjugated Δ^4-3-ketocorticosteroids are likely potentially biologically active, and the absence of *free* biologically active material in these pre- and postoperative gastrectomy samples does not necessarily indicate adrenocortical insufficiency in this patient.

In certain instances (Tables 1, 2 and 4) it is shown that when free INHR material is virtually absent, the level of conjugated INHR material is elevated, and vice versa; similarly, when the level of INHR conjugates decreases, the level of PSR conjugates increases, and vice versa. This suggests that under certain conditions (after ACTH infusions, gastrectomy, burns, and in terminal cancer) there are variations in the pattern of adrenocortical steroids. Consequently, in order to obtain a valid comparison of adrenocortical responses between patients, it is necessary to determine both the free and the conjugated PSR and INHR steroidal materials. Examples of the need for such multiple determinations are illustrated in Tables 3 and 4.

In Table 4 the instances of low or virtually-absent free and conjugated INHR material are explainable on the basis that relative adrenocortical insufficiency or even failure may exist without clinical manifestations. This clinical condition has actually been observed in 2 totally adrenalectomized patients showing no gross signs of adrenocortical insufficiency; moreover free and conjugated PSR and INHR material had practically disappeared from the peripheral plasma three days after withdrawal of hydrocortisone. Apparently a relatively normal clinical state for at least short periods of time without stress is compatible with virtual absence of active circulating adrenocortical steroids in the peripheral blood. Cachexia is known to be one condition associated with a definite decrease in pituitary function, manifested by a suppression of ovarian and testicular, as well as adrenocortical activity. The virtual though temporary disappearance of active

steroids observed in the cachectic cancer-bearing patients (Table 4) in our study may be interpreted as a variable pituitary (ACTH) insufficiency, inasmuch as all these patients had intact adrenals.

These observations are being continued on blood from patients undergoing elective and emergency gastrectomies. Large quantities of fresh postmortem blood are also being obtained from terminal cancer patients in order to facilitate isolation and chemical identification of certain of these "abnormal" metabolites.

SUMMARY

Potentially biologically active corticosteroids (Δ^4-3-ketocorticosteroids), both free and glucuronic-acid conjugated, have been measured by a chemical (isonicotinic acid hydrazide) reaction in the peripheral plasma of patients before and after infusion of ACTH, before and after gastrectomy, after severe burns, and in terminal mammary cancer. The concentrations of free and conjugated Porter-Silber (17-hydroxycorticosteroids) and alkaline blue-tetrazolium reducing (alpha-ketol corticosteroids) materials have also been determined. By the use of these chemical reactions, confirmatory evidence has been obtained of increased adrenocortical secretion after ACTH infusion, gastrectomy and burns.

As much as 50 per cent of the free Porter-Silber reactive material is not measured as Δ^4-3-ketocorticosteroids by the INH reaction, suggesting that after surgical operation or trauma or in far-advanced mammary cancer the free Porter-Silber reactive material in peripheral blood does not represent biologically active steroids alone, but also certain unconjugated inactive metabolites.

In the peripheral blood of certain patients it has been observed that the level of free isonicotinic-acid hydrazide reactive material is in excess of the level of free Porter-Silber reactive material or even of free alkaline blue-tetrazolium reactive material. This finding suggests the presence of certain 20-hydroxylated steroids containing the Δ^4-3-keto grouping.

The marked variations in the metabolic pattern of adrenocortical steroids as revealed by these studies emphasize the fact that, in order to obtain a valid appraisal of adrenocortical response, it is necessary to determine both free and conjugated Porter-Silber reactive and INH reactive materials in the peripheral blood. In some instances it may be desirable to determine also the free blue-tetrazolium reactive material.

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URINARY METABOLITES OF CORTISOL IN ADRENAL INSUFFICIENCY AND IN PITUITARY EUNUCHOIDISM

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PREVIOUS to the development of the highly specific replacement therapy now available for the treatment of Addison's disease, the average life expectancy of such patients was about one and a half years (1). Nevertheless, patients with the classical signs and symptoms of the condition were observed who survived for much longer periods of time. On clinical grounds it appeared that in such patients a small remnant of functioning adrenal tissue sufficed to provide the small quantity of adrenal hormones necessary to permit survival, although often in a condition of semi-invalidism. The diagnosis of Addison's disease, even when beyond doubt on clinical grounds, cannot always be confirmed by characteristic changes in the levels of serum electrolytes, nor in the urinary excretion of steroids derived from the secretions of the adrenal cortex. In such cases the patient may have a small amount of functioning adrenocortical tissue and, if untreated, may later pass into a dangerous state of acute adrenal insufficiency either because of the increased adrenal hormone requirements associated with an incidental infection, or because of the final destruction or atrophy of all the adrenal cortex. With the ready availability of cortisone for the treatment of adrenal insufficiency, the diagnosis in such cases is most important. Laidlaw and associates (2) referred to 7 patients with definite clinical evidence of Addison's disease whose urinary excretion of 17-hydroxycorticoids (as estimated by the method of Reddy, Jenkins and Thorn (3)) was within the normal range. The urines of 5 of these patients were subsequently shown by paper chromatography to contain tetrahydrocortisone and tetrahydrocortisol after glucuronidase hydrolysis.

The present paper describes 3 patients with some of the clinical features of Addison's disease whose urine contained appreciable amounts of adrenocortical hormones or their metabolites. The administration of cortico-

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tropin (ACTH) was followed by a fall in adrenocortical steroid excretion to negligible amounts, although this was sometimes preceded by a temporary and slight augmentation. These results contrast markedly with those obtained when corticotropin was administered to a fourth subject with pigmentation but without other clinical evidence of Addison's disease and to 2 subjects with eunuchoidism of pituitary origin.

CASE REPORTS

Case 1

A single, 46-year-old woman was admitted in June 1954 for investigation of pigmentation of six months' duration. Apart from appendicectomy in 1932 she had always been healthy. There was no history of contact with tuberculosis, although 2 maternal uncles had died of the disease some years previously. On examination she was a normally developed woman of medium height, weighing 51.3 Kg. There was moderate pigmentation of the face, neck, creases of hands, axillae and waist area. There were some patches of pigment on the buccal mucosa. The distribution of hair was normal. Blood pressure was 145/80 mm. Hg. There were no other significant findings.

Radiographs of the chest and abdomen were normal, as was an excretion pyelogram. Blood picture—hemoglobin level 102 per cent, total white cell count 7,500 (91 per cent polymorphs, 5 per cent lymphocytes, 3 per cent eosinophils and 1 per cent monocytes). The level of serum sodium was 140 mEq./L., potassium 4.0 mEq./L., chloride 103 mEq./L., plasma bicarbonate 24 mEq./L. and blood urea 28 mg./100 ml. During a water loading test, 70 per cent of the load was excreted within four hours. The insulin sensitivity test showed a normal fall in the level of blood sugar and hypoglycemic responsiveness. The urinary excretion of 17-ketosteroids was 1.8 mg. per twenty-four hours. The urinary excretion of adrenal steroids was measured by the methods described subsequently, and yielded normal results, although cortisone excretion was at the lower limit of normal (Fig. 1).

The patient was given 40 mg. of Armour ACTH-gel (corticotropin) twice daily for four days by intramuscular injections. Adrenal steroid excretion remained low, and even when an intravenous infusion of 40 mg. of ACTH in physiologic salt solution was given during eight hours on the fifth day, cortisone, hydrocortisone and Reichstein's compound U could not be detected, although the excretion of tetrahydrocortisone which had fallen to half its original value was unchanged. At this time, the urinary neutral 17-ketosteroids were 3.9 mg. per twenty-four hours. The patient was discharged at her own request without treatment and re-admitted in September 1955 for further assessment.

On her second admission her skin was darker (following sun-bathing) and, in particular, there was more marked pigmentation of the buccal mucosa, gums and sides of the tongue. The blood pressure was 130/80, menses were regular, the level of serum electrolytes was normal, and 17-ketosteroid excretion was 2.7 mg. per twenty-four hours. On this occasion the excretion of tetrahydrocortisone and tetrahydrohydrocortisone was within normal limits (Fig. 1). No Reichstein's compound U or hydrocortisone could be detected in the urine and the excretion of cortisone was only 10 μg. per twenty-four hours. She was again given ACTH-gel, 40 mg. twice daily for four days, followed by an infusion as before. On this occasion the intramuscular injection of ACTH led to the disappearance of cortisone and hydrocortisone from the urine, although the excretion of Reichstein's compound U was increased. Tetrahydrocortisone excretion at first decreased, then increased on the fourth day and fell to the lowest value on the fifth day.

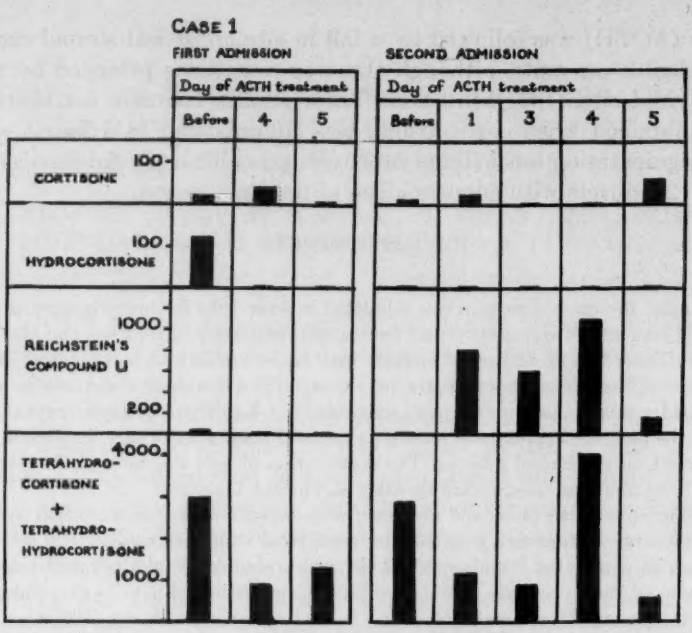

FIG. 1. Effect of ACTH on urinary adrenal steroids in partial Addison's disease (Case 1).

On the fifth day, when the intravenous infusion was given, a small quantity of cortisone was excreted, but no hydrocortisone; the excretion of the compound U and the tetrahydro derivatives was markedly reduced. After the infusion of corticotropin had continued for five hours and about 25 mg. had been given, the patient complained of feeling cold, became restless and nauseated, and began to perspire. The pulse rate rose to 116 per minute and the temperature to 100° F. The blood pressure dropped to 94/74. She vomited three times. The serum sodium level dropped to 121 mEq./L. She was regarded as passing into a state of adrenal crisis and was given 50 mg. of cortisone by mouth; within four hours she was subjectively well and the blood pressure had risen to 120/70.

Case 2

A 47-year-old woman was admitted in August 1955 for investigation of pigmentation and two short attacks of indigestion, mild diarrhea and sore throat without dysphagia which had occurred eight months and six weeks respectively before admission. She had always tanned easily in the summer months but over the previous two years the tan had remained even during the winter. She had been subject to mild asthma in the summer for many years. The menopause had occurred three years before admission. She denied lassitude or any other symptoms. There was no history of tuberculosis or familial endocrine disorder.

On examination she was a well-nourished woman of average build with generalized moderate pigmentation, most noticeable on the face, creases of the skin, axillae, elbows, groins, inside of the lips and the buccal mucosa, as well as at pressure points of her

corset and brassiere. The distribution of hair was normal. Blood pressure was 115/80. There were no other significant findings.

A blood film showed mild neutropenia, with a total white cell count of 3,300 (58 per cent polymorphs, 14 per cent eosinophils, 27 per cent lymphocytes and 1 per cent monocytes). The erythrocyte sedimentation rate was 7 mm. per hour (Westergren). The hemoglobin level was 90 per cent. Radiographs of the chest and abdomen showed no abnormality. The concentration of serum sodium was 152 mEq./L., potassium 4.3 mEq./L., chloride 109 m.Eq./L., plasma bicarbonate 24 m.Eq/L. and blood urea 25 mg./100 ml. During a water loading test, 22 per cent of the load was excreted within four hours. The Kepler-Robinson-Power index was 3. The 17-ketosteroid excretion

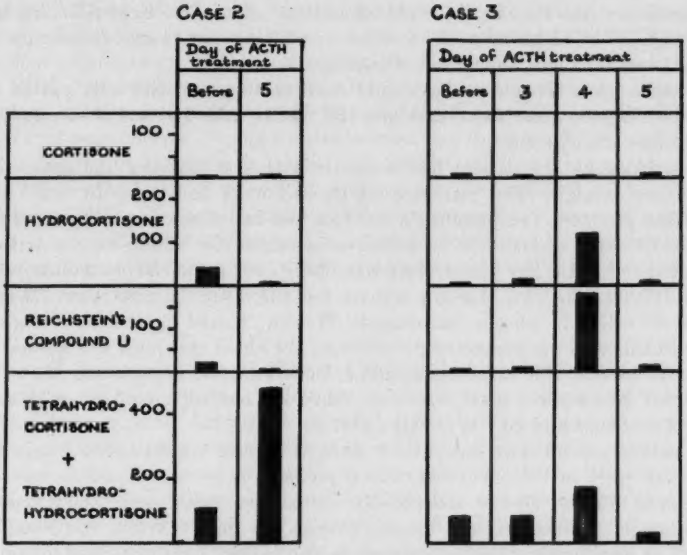

FIG. 2. Effect of ACTH on urinary adrenal steroids in partial Addison's disease (Cases 2 and 3).

was 0.7 mg. per twenty-four hours. Insulin sensitivity was normal. The urinary 24-hour excretion of adrenal steroids was low; cortisone was absent but small amounts of hydrocortisone, Reichstein's compound U and tetrahydrocortisone were present (Fig. 2).

The patient was then given ACTH-gel intramuscularly, 40 mg. twice daily for four days. There were no untoward symptoms and on the fifth day an intravenous infusion was given, as in Case 1. Measurements of adrenocortical steroid excretion on the day upon which the infusion was given showed that, except for a very small increase in tetrahydrocortisone, the excretion of the other steroids had dropped to undetectable amounts. At the end of the infusion the patient complained of feeling generally ill and nauseated. She was given cortisone and salt supplements but the salt capsules accentuated her nausea and she began to vomit. The salt capsules were reduced in number and she improved clinically and is now maintained extremely well on 25 mg. of cortisone daily by mouth with some extra salt.

Case 3

A woman aged 50 was first admitted in April 1953 with a history of pigmentation for the preceding three years and increasing tiredness for twelve months. For three months she had occasionally experienced morning nausea but had never vomited. She had a past history of tuberculosis of the right sacro-iliac joint with a psoas abscess in 1929. On examination she had mild though definite pigmentation of the face and exposed parts and in her vaccination scar, but not in her mouth. Blood pressure was 130/80. There were no other abnormal findings.

The blood picture and the levels of serum electrolytes, plasma bicarbonate and blood urea were normal. The erythrocyte sedimentation rate was 28 mm. per hour (Westergren). Radiographs of chest and abdomen showed no abnormality apart from an inactive old tuberculous infection in the right sacro-iliac joint. The Kepler-Robinson-Power index was 4.2. The 17-ketosteroid excretion was 1.0 mg. per twenty-four hours. During an ACTH test (4) there was no fall of eosinophils.

The patient was thought to have mild Addison's disease with only partial adrenocortical insufficiency. She was discharged and told to take 5 grams of salt daily and to remain under observation.

Two weeks later the patient had a sudden attack of diarrhea and generalized abdominal pain at night. The next morning she felt weak and began to vomit although the diarrhea stopped. The patient's 2 children had had minor bowel upsets three days earlier which were thought to be infective in origin. On admission the patient was dehydrated and weak. Her temperature was 104° F, pulse rate 120 per minute, and blood pressure 100/60. The level of serum sodium was 133 mEq./L., potassium 3.9 mEq./L., chloride 109 mEq./L., plasma bicarbonate 29 mEq./L. and blood urea 26 mg./100 ml.

An adrenal crisis was suspected even though the blood urea level was normal and the patient was treated with intravenous saline, Eucortone and bicarbonate; then cortisone was started. She made a good recovery. When discharged, maintenance therapy was 25 mg. of cortisone and 10 Gm of salt daily.

The patient attended an out-patient clinic at regular intervals. She was in excellent health until early in 1955, when her blood pressure on several occasions was found to have risen to 170–180/95–110. Her previous history was reconsidered and it was thought that, although the diagnosis of Addison's disease was almost certain, confirmatory tests had been by no means unequivocal; therefore, the patient was readmitted for assessment of adrenocortical function.

On examination she was only slightly tanned, without any pigmentation of the mouth or buccal mucosa. Axillary hair was scanty. The resting blood pressure was 145/75. There were no other abnormal findings. All treatment was stopped for one week before investigations were commenced.

Radiographs of the abdomen showed no adrenal calcification. The levels of serum electrolytes were normal. During a water loading test, only 12 per cent of the load was excreted within four hours. Urinary 17-ketosteroid excretion was 0.4 mg. per twenty-four hours. Analysis of the urine for adrenocortical steroids showed very low but significant traces of cortisone, Reichstein's compound U, and tetrahydrocortisone. Hydrocortisone could just be detected.

The patient was given ACTH-gel intramuscularly followed by an intravenous infusion. Although a significant quantity of hydrocortisone and of compound U was excreted on the fourth day of administration of the gel, it fell to only 10 μg. during the day she was given the intravenous corticotropin, and at this time the other steroids remained barely measurable. Urinary 17-ketosteroid excretion was now 0.2 mg. per twenty-four hours.

Case 4

A man aged 44 was admitted in September 1955 with a nine-month history of progressive loss of weight amounting to nearly 28 pounds, slight loss of energy, lassitude, irritability and weakness. During the preceding six months he had suffered from isolated attacks of nausea generally followed by vomiting. This was unrelated to meals and occurred about once in every three or four weeks. He had always been swarthy but thought he might have become more pigmented recently. Apart from jaundice in youth, pneumonia at the age of 21 years, and a right nephrectomy for a renal calculus in 1944, he had always been healthy. There was no past or family history of endocrine disorder but 2 siblings had suffered from pulmonary tuberculosis.

The only significant finding on examination was mild generalized pigmentation, including the "bathing trunk" area, creases of palms and axillae, but not the mouth. The blood pressure was consistently low—in the neighborhood of 100/70. Apart from varicose veins, there were no other abnormal physical findings.

The blood picture, chest roentgenogram, intravenous pyelogram, barium-meal examination, and determinations of the levels of serum electrolytes, plasma bicarbonate and blood urea were normal. During a water loading test, there was 78 per cent excretion within four hours. An intravenous insulin sensitivity test yielded normal results. The urinary 17-ketosteroid excretion was 4.2 and 7.8 mg. per twenty-four hours on two different days. Although normal amounts of tetrahydrocortisone were excreted in the urine, only traces of hydrocortisone and Reichstein's compound U, and only 24 μg. of cortisone were present. Nevertheless, during treatment with ACTH the excretions of all these compounds were greatly increased.

Case 5

A man aged 43 was referred to the hospital in June 1954 for study of endocrine status. He presented as a case of dwarfism with progeria. His early development up to the age of 5 years had been normal. Thereafter his rate of growth and development decreased markedly and although he continued to grow he did not attain adult proportions. Puberty did not occur and he continued to look like a little boy until two or three years before admission, when his face became aged and lined. He was of average intelligence, sensitive, shy, and very anxious to please. At times he suffered from mood swings, ideas of grandeur and mild paranoid reactions, which were considered the result of environmental strain on a predisposed personality rather than related to his physical condition. Libido was absent and he had never felt any attraction to the opposite sex, or experienced sexual phantasies. There had never been any erections or emissions. There was nothing else relevant in his past or family history.

On examination his face looked old and wrinkled, with a soft transparent skin and thin eyebrows. The scalp hair was of fine texture; otherwise body hair was absent. He was slim and small and his proportions were eunuchoidal, *i.e.*, height 61 inches, arm span 63 inches, vertex to pubis 29 inches, and pubis to ground 32 inches. His weight was 96 pounds. The larynx was undeveloped and the voice high pitched. The penis was infantile and the scrotum small. The prostate gland was small. The thyroid gland was not palpable. Blood pressure was 104/64. There were no other abnormal findings on clinical examination.

He was mildly anemic with a hemoglobin level of 73 per cent. Otherwise the blood picture, the spinal fluid, and the concentrations of serum electrolytes and uric acid were within the normal range. Insulin sensitivity was normal, as was hypoglycemic responsiveness. The B.M.R. was −31 per cent and the serum cholesterol level was 200 mg. per 100 ml. Thyroidal radioiodine uptake was at the lower limit of normal, and the plasma

protein-bound I^{131} level was within the normal range. The electrocardiogram showed low voltage in all leads, suggestive of hypothyroidism. Following a water load the patient excreted less than 20 per cent in four hours, and the Kepler-Robinson-Power index was 4.9. A radiograph of the skull showed a small pituitary fossa. In radiographs of the skeleton, it was evident that epiphyseal union was delayed, the bone age corresponding to 16 years. Testicular biopsy showed simple tubules lined by a single layer of cuboidal epithelium without any evidence of spermatogenesis and no interstitial cells. Urinary gonadotropin excretion was less than 6 mouse units per twenty-four hours.

Before treatment with ACTH only negligible quantities of adrenal steroids were excreted, but after the ACTH test the excretion of all the adrenal steroids measured had increased to very high values (Fig. 3).

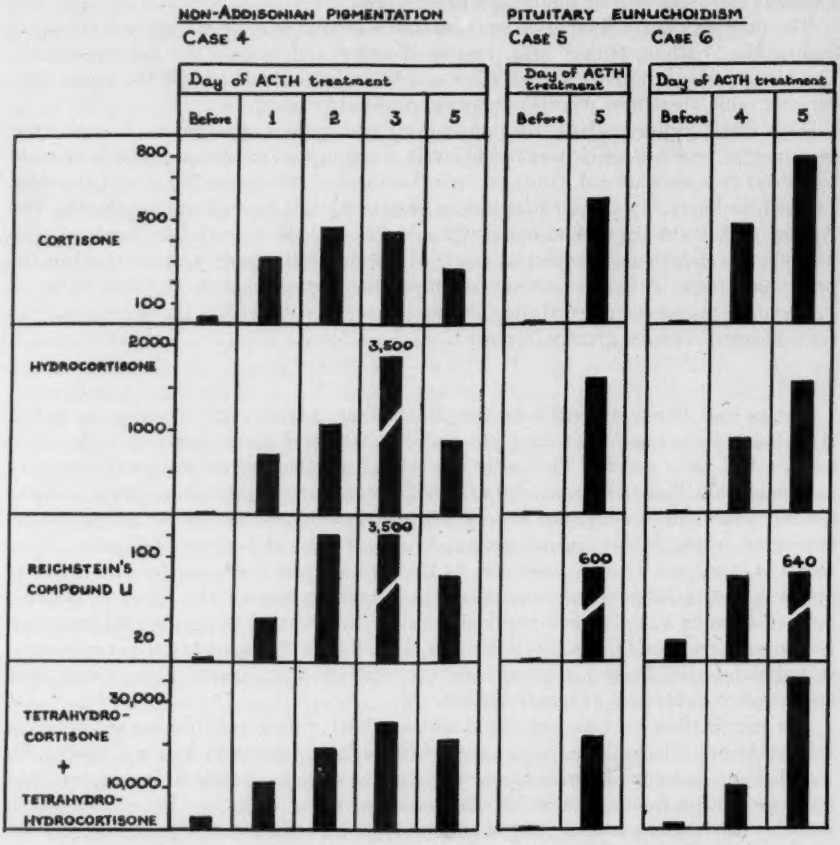

FIG. 3. Effect of ACTH on urinary adrenal steroids (Cases 4, 5 and 6).

Case 6

A man aged 23 was referred to the hospital in May 1954, on account of hypogonadism. Except for a period of headaches and vomiting at the age of about 12 years, he had been

in good health and had developed normally. However, puberty failed to occur and at about the age of 17 years he put on weight about his chest and hips, giving him a feminine body configuration. He was then given a course of testosterone injections, 25 mg. thrice weekly for six months, by his own doctor; this caused some growth of hair in the pubic region and to a lesser extent in the axillae and beard areas. The voice became a little lower and the penis enlarged. Following the injections he experienced an occasional erection but no emissions. Libido remained absent and he preferred male to female company. There were no other relevant findings in his past history or family history.

The blood picture, and the levels of serum electrolytes, serum cholesterol, blood urea, and blood uric acid were within the normal range. Results of glucose tolerance and insulin sensitivity tests were also normal. An electrocardiogram showed low voltage in all leads, compatible with a diagnosis of myxedema. The B.M.R. was −25 per cent, but the thyroidal uptake of radioactive iodine was within normal limits. During a water loading test, only 19 per cent of the load was excreted in four hours and, the Kepler-Robinson-Power index was 5.2. Radiographs of the skull showed considerable enlargement of the sella turcica, but the visual fields were normal. Radiographs of the skeleton showed that epiphyseal union was delayed, the bone age corresponding to 14 years. Urinary gonadotropin excretion was less than 6 mouse uterine units per twenty-four hours. When the patient was given cortisone in order to study the effect of replacement therapy, polydipsia and polyuria developed, with a urine flow of up to 5 liters per day—which suggested the additional diagnosis of diabetes insipidus. This was confirmed by the results of an intravenous saline infusion test.

Before treatment with ACTH only negligible quantities of adrenal steroids were excreted, but after the standard ACTH test the excretion of all adrenal steroids measured had increased to very high values. The 24-hour urinary 17-ketosteroid excretion increased from 0.07 mg. before ACTH to 4.7 mg. after ACTH.

LABORATORY METHODS

Analyses of serum were carried out by standard laboratory procedures. Total neutral 17-ketosteroids were measured on 24-hour urine specimens by a method similar to that recommended by the Medical Research Council Committee on Endocrinology (5). Adrenocortical steroids were measured by the method of de Courcy, Bush, Gray and Lunnon (6) modified as described by Gray, Lunnon, Pond and Simpson (7). In this method, cortisone, compound F and Reichstein's compound U (Δ^4-pregnene-17α,20β-21-triol-3,11-dione) are regularly present in the free steroid fraction. Tetrahydrocortisone and tetrahydro compound F are present in the conjugated fraction, the former in much greater amount than the latter. Semiquantitative estimation of each of these compounds was made, as described by Gray and associates (7).

Insulin sensitivity was investigated by the method of Fraser and Smith (8). The ability of the patients to eliminate a water load was determined by the volume of urine excreted within four hours after giving water in a dose of 20 ml. per Kg. of body weight. The Kepler-Robinson-Power test was carried out as recommended by the original authors (9).

DISCUSSION

Cases 2 and 3 are unequivocal examples of adrenal insufficiency but the diagnosis in Case 1 is open to discussion. In this instance, the only symptom of which the patient initially complained was pigmentation and, except for the urinary steroid values before and after ACTH, there were no other clinical features of Addison's disease, nor was the diagnosis supported by

the levels of serum electrolytes or the results of other laboratory investigations. At the time of this patient's first admission, the excretion of adrenal steroids was within normal limits, although the excretion of 17-ketosteroids was significantly low (1.8 mg. per twenty-four hours). After four days of ACTH, no hydrocortisone could be detected in the urine, the excretion of cortisone and Reichstein's compound U was little changed, and tetrahydrocortisone and tetrahydrohydrocortisone excretion had fallen to about a third of the initial values. The possibility of an inactive preparation of ACTH appeared to be excluded by the results obtained when a different preparation of this hormone was given intravenously on the fifth day; no cortisone, hydrocortisone or Reichstein's compound U could be detected and the quantity of reduced derivatives increased slightly to only half the initial value. Qualitatively similar results were obtained fifteen months later. Before ACTH was given this time, greatly reduced amounts of cortisone, hydrocortisone and Reichstein's compound U were excreted with relatively normal quantities of the tetrahydro compounds. Administration of ACTH resulted in a substantially increased excretion of Reichstein's compound U, but no significant change in the tetrahydro compounds. These responses to ACTH were very different from the great increases observed in all the measured adrenal steroids in Cases 4, 5 and 6, in which presumably there was no deficiency of adrenal tissue. These findings in Case 1, together with the apparent Addisonian crisis (supported by the plasma electrolyte values), seem to indicate that in this patient sufficient adrenal tissue was present to produce a limited amount of adrenocortical hormone but, since the gland was incapable of responding to ACTH, it was presumably excreting the maximum amount of hormone of which it was capable.

In Cases 2 and 3 the diagnosis was more obvious from the ordinary laboratory findings, without the special investigation of the response of the urinary metabolites of cortisol to administration of ACTH. In Case 2 the patient presented with mild pigmentation and two short vague attacks of indigestion, and in Case 3 with pigmentation and tiredness. In both cases the 24-hour 17-ketosteroid excretion was reduced and there were abnormalities in the water loading response and Kepler-Robinson-Power index, but insulin sensitivity and hypoglycemic responses were within normal limits. In these 2 cases the 24-hour urinary excretion of adrenocortical steroids was low before treatment with ACTH. After five days of ACTH therapy, including a final intravenous dose, the only adrenocortical steroid material detected in the urine was a small quantity of the tetrahydro compounds in Case 2 and minute quantities of both reduced and unreduced compounds in Case 3.

In Case 4 the patient also presented with pigmentation and loss of energy, and his blood pressure was consistently low. Nevertheless the re-

sults of the usual laboratory tests were normal, except that urinary 17-ketosteroid excretion was at the lower limit of the normal range. Analysis of the urine for adrenal steroids before administration of ACTH revealed that, apart from normal amounts of tetrahydrocortisone, only traces of other adrenocortical steroids were present. On the basis of these findings, the diagnosis seemed to be one of partial Addison's disease, similar to that made in Cases 1, 2 and 3. However, the response of the urinary adrenocortical steroids after administration of ACTH excluded this diagnosis beyond all doubt.

In Cases 5 and 6 there was evidence of a deficiency of pituitary tropic hormones and a reduced or absent function of the target organs. Adrenal function was markedly impaired, as indicated by the low 17-ketosteroid excretion, the lack of water diuresis after a water load, and the low Kepler-Robinson-Power index comparable with that observed in Cases 1, 2 and 3. In Cases 5 and 6, however, the enormous response of the urinary adrenal steroids following stimulation by corticotropin was distinctly different from that observed in the other 3 cases.

The evidence, therefore, seems conclusive that Cases 1, 2 and 3 may be regarded as cases of partial Addison's disease in which a small amount of surviving adrenal tissue permitted the excretion of sufficient hormone to support life, and that in these cases stimulation by ACTH and probably by other factors such as infection resulted in exhaustion of the adrenal cortex and the disappearance of cortisone and hydrocortisone from the urine.

Nordin (10) reported a case of Addison's disease diagnosed when the patient was 26 years old, with subsequent apparent recovery. When the patient was re-investigated at the age of 48 years, the results of all laboratory tests were within normal limits except for a somewhat low 17-ketosteroid excretion similar to that in Case 1; urinary cortisone and hydrocortisone excretions (measured by the method of Cope and Hurlock (11)) were normal, but there was no rise following administration of corticotropin as in the present cases. Perloff *et al.* (12), Christy, Wallace and Jailer (13), Laidlaw *et al.* (2) and Bayliss (14) all described cases of Addison's disease with normal 17-hydroxycorticoid levels in blood or urine, but with no increase after corticotropin stimulation. Laidlaw *et al.* (2) confirmed by paper chromatography that tetrahydrocortisone and tetrahydrohydrocortisone were present in the urine. However, Eik-Nes *et al.* (15) observed appreciable, though subnormal, increases in the level of plasma 17-hydroxycorticoids within two hours of the start of an infusion of corticotropin in 2 out of 6 patients with Addison's disease. These investigations were carried out on plasma during only short periods of adrenocortical stimulation and are therefore not comparable with the present results. There is some similarity insofar as in the present investigation Cases 1 and 3 also

showed transient increments in oxysteroid excretion, suggesting the presence of an adrenal remnant capable of a limited response to exogenously administered corticotropin. This is also supported by the gain in weight of the patient in Case 1 during treatment with ACTH-gel.

Since patients with Addison's disease may have normal plasma levels and urinary excretion of adrenocortical hormones and, conversely (as in Case 4), normal adrenal reserve may be present with low urinary excretion of cortical hormones, the diagnosis of partial Addison's disease can only be made with certainty by assessing the response of the adrenal cortex to corticotropin. However, stimulation by means of corticotropin for measurement of adrenocortical reserve in patients suspected of Addison's disease carries a considerable risk and should be carried out only when the clinical and other laboratory findings are inconclusive. Following the intravenous infusion of corticotropin after four days of intramuscular administration of ACTH-gel, in Case 2 and on the second occasion in Case 1, there were adverse reactions suggestive of incipient acute adrenal insufficiency. In both cases these reactions occurred several hours after the start of the infusion and were characterized by fever, malaise, hypotension, sweating, nausea, and in Case 1 by vomiting.

It is interesting to observe that when adrenal steroids are present in small amounts in the urine the proportions of the individual steroids are just as irregular and unpredictable as they are in the normal individual and in the subject with Cushing's disease. Considering the steroids which are excreted in the free state—in some cases cortisone is the predominant steroid and in other cases, hydrocortisone. Tetrahydrocortisone and tetrahydrohydrocortisone are almost always present; they may even be observed in cases of frank Addison's disease, though in very small amounts. It is interesting that in Case 1 substantial amounts of these reduced compounds were present in the urine, though they were not significantly augmented after administration of ACTH, and though the quantities of urinary free steroids were insignificant. It seems as if the agents which determine the proportions of free and conjugated steroids and the proportions of individual steroids in these fractions are regulated by completely unknown factors. Indeed there seems to be some evidence that the clinical condition is related to the quantity of free steroids in the urine (perhaps reflecting the level of active hormones in the blood). It is still not known whether the adrenocortical hormone produces its physiologic effect on the tissues directly or whether this effect is linked with a chemical transformation.

SUMMARY

1. Three cases of pigmentation due to partial Addison's disease are described, together with a case of pigmentation not associated with Addison's disease and 2 cases of hypo-activity of the adrenal cortex secondary to anterior pituitary insufficiency.

2. Significant quantities of cortisol or its metabolites were excreted in the urine of the first 3 patients, but the amounts were not increased after stimulation with corticotropin.

3. In the 2 eunuchoid subjects corticotropin greatly augmented the urinary excretion of cortisol and its metabolites. Before corticotropin was given, the excretion was similar to that in the 3 cases of partial Addison's disease.

4. The proportions of individual steroids excreted in the urine were irregular and unpredictable, as they are in normal subjects and in patients with Cushing's disease.

5. The dangers of administering corticotropin to patients with Addison's disease are emphasized.

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URINARY KETOSTEROID EXCRETION PATTERNS IN CONGENITAL ADRENAL HYPERPLASIA*

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THE present concepts of the aberrant mechanisms in congenital adrenal hyperplasia have recently been summarized by Jailer *et al.* (1), Bongiovanni and Eberlein (2) and Dorfman (3). The basic defect is thought to be the inability of the adrenal cortices to produce adequate amounts of hydrocortisone. The low levels of blood hydrocortisone lead to a compensatory increase in secretion of corticotropin by the anterior pituitary gland, and consequent adrenal hyperplasia. The hyperplastic adrenal cortices, unable to effect the adequate synthesis of hydrocortisone, secrete, among other steroids, androgen(s) or androgen precursor(s) which bring about the clinical picture.

The identity of the androgenic compounds which induce the signs of virilism is uncertain. Dorfman (4) believed that the androgenicity could be attributed to the C_{19} adrenal steroids such as 4-androstene-3,17-dione (androstenedione) and 11-hydroxyandrostene-3,17-dione (11-hydroxyandrostenedione), whereas Jailer *et al.* (1) held that the intermediates such as 17-hydroxyprogesterone and 21-desoxyhydrocortisone or their metabolites such as androsterone and 11-hydroxyandrosterone, could account for the virilization.

In this study an attempt has been made to identify the patterns of urinary ketosteroid excretion in 5 cases of congenital adrenal hyperplasia.

METHODS

Ketosteroids were measured in the urines from 5 untreated patients with congenital adrenal hyperplasia—3 female pseudohermaphrodites[1] aged 15, 18 and 34 years, and 2 boys with macrogenitosomia praecox[2] aged $4\frac{1}{2}$ and $7\frac{1}{2}$ years. For comparison, keto-

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[1] Urines obtained from patients of Dr. Elizabeth K. Smith, Associate Professor of Obstetrics and Gynecology, University of Washington School of Medicine, Seattle. Diagnoses confirmed at laparotomy.

[2] Urines obtained from patients of Dr. Horace Thuline, Children's Orthopedic Hospital, Seattle. These patients were successfully treated with cortisone.

steroids were measured similarly in the urines from 4 normal individuals—2 women aged 23 and 29 years, and 2 boys, both 6 years old.

All urines were treated in the following manner. A sufficient volume to ensure the proper amount of ketosteroid (10–20 mg.) on an alumina chromatographic column was refluxed for ten to fifteen hours according to the method of Jensen and Tötterman (5). After ether extraction (twice with 3 volumes of ether), the residual urine was incubated with β-glucuronidase.[3] Conditions for enzymatic hydrolysis were pH 5.0 at 37.5° C. for seventy-two hours using 360 units of enzyme per cc. of buffered urine (1/15 M sodium acetate). After ether extraction, any remaining conjugates were hydrolyzed by the method of Drekter *et al.* (6). The ether extracts obtained after β-glucuronidase incubation and after treatment with heat and acid were combined, since the methods release like steroids from their conjugates (7). The ketonic Girard fraction from the combined extract was obtained, using the method of Pincus and Pearlman (8). The acetates formed from this procedure (9) were hydrolyzed by treatment with 3 per cent ethanolic potassium hydroxide overnight at room temperature (10). The steroids were recovered from the alcoholic potassium hydroxide by partitioning between ether and water. The ether extracts were dried over anhydrous sodium sulfate and evaporated to dryness.

Fractionation of the urinary ketosteroids was effected by alumina chromatographic procedures which were modifications (7) of the method of Dingemanse *et al.* (11), utilizing as eluants benzene-ethanol mixtures. The combination of extracts obtained after treatment with β-glucuronidase and heating with acid was chromatographed separately from the extract obtained after refluxing without acid. The latter extract, containing principally the 3-β-hydroxyketosteroids, was chromatographed on an alumina column of different dimensions and alumina weight, since the amount of ketosteroids obtained by this hydrolysis is a smaller variable fraction of the total urinary ketosteroids (4 to 36 per cent) (7).

The ketosteroids, fractionated into the 8 peaks which comprise the alumina chromatogram, were identified by comparison of the infrared spectra of chromatographic fractions with that of known pure steroids[4] or published spectra (12, 13). Identification and relative estimation of individual ketosteroids in mixtures were made on the basis of the location and intensities of characteristic absorption bands of the steroids eluted in these fractions. Quantitative estimation of the ketosteroids identified was carried out on the basis of the Zimmermann chromogenic titer by the method of Drekter *et al.* (6), using dehydroisoandrosterone as standard. Pregnanetriol determinations on the urines of the 3 female pseudohermaphrodites were performed in Dr. Elizabeth K. Smith's laboratory, using the method of Bongiovanni and Clayton (14).

RESULTS

The individual 24-hour urinary ketosteroid excretion patterns as found in the 5 patients with congenital adrenal hyperplasia and in 4 normal subjects are shown in Table 1.

The mean titers of the individual ketosteroids in the 4 comparative groups are shown as bar graphs in Figure 1. There was considerable variation in the titer of the individual ketosteroids among the patients, but in

[3] Warner-Chilcott's Ketodase.

[4] Acknowledgment is made of contributions of steroids by Ciba Pharmaceutical Products, Merck & Co., Schering Corporation, and Syntex, S. A.

TABLE 1. URINARY KETOSTEROID EXCRETION PATTERNS IN NORMAL SUBJECTS AND IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

(mg./24 hrs.)

Urinary steroids	Congenital adrenal hyperplasia					Normal subjects			
	K.E.B. ♀, 34	N.N. ♀, 18	J.H. ♀, 15	R.C. ♂, 7½	J.S. ♂, 4½	T.T. ♂, 6	L.H. ♂, 6	A.D. ♀, 23	B.H. ♀, 29
Dehydroisoandrosterone	3.9*	†			3.7			.45	.2
Androsterone	9.6	4.1	3.5	1.4	4.3	.03	.10	2.8	2.0
Etiocholanolone	4.6	3.5	5.6	.2	1.6	.06	.13	3.1	2.3
Pregnanolone	.8	.5	2.2	.5	.7			.2	.1
11-Hydroxyandrosterone	4.9	4.1	3.3	1.2	2.0	.03	.07	.47	.25
9(11)-Androstenolone‡	1.0	1.0	.7	1.6	.5	.03	.04	.14	.16
11-Hydroxyetiocholanolone						.04	.05	.6	.2
11-Ketoetiocholanolone	1.8	1.7	1.0	1.1	1.8	.05	.06	.4	.4
11-Ketoandrosterone					.2	.01	.03		.1
11-Ketopregnanolone			.6	.8		.01	.05	.2	
17-Hydroxypregnanolone§	1.6	1.8	1.2						
Pregnanetriol‖	24.8	51.7	6.5	not det'd	not det'd	not det'd	not det'd	not det'd	not det'd
Total ketonic (chromatogram)	33.8	22.0	21.5	10.1	20.9	.5	.78	9.2	6.9
Ketonic identifiable	28.2	16.7	16.1	6.8	14.8	.26	.53	8.4	5.7

* Plus 3,5-cycloandrostan-6β-ol-17-one.

† Blank spaces indicate amounts too small to be identified.

‡ 9(11)-Androstenolone is a chemical artifact produced by action of heat and acid on 11-hydroxyandrosterone (15).

§ As 3α,17α-dihydroxy-17β-methyl-D-homoetiocholan-17a-one (16).

‖ Determinations by Dr. Elizabeth K. Smith.

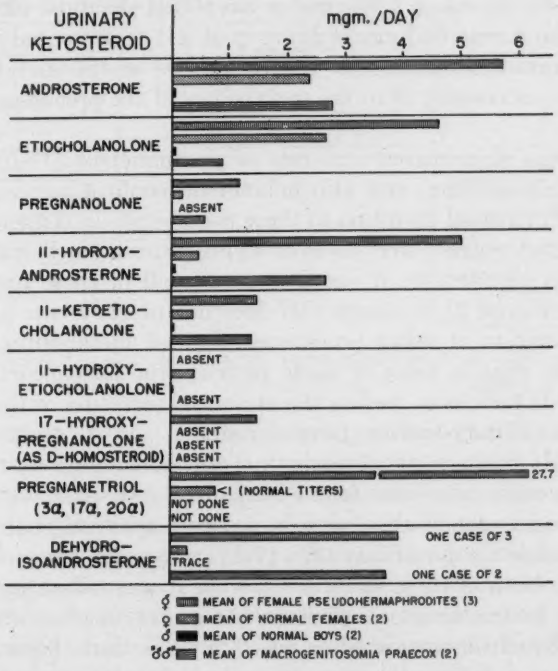

FIG. 1. Mean ketosteroid titers in patients with congenital adrenal hyperplasia as compared to normal subjects.

no case was there an overlap in titer when compared with normal subjects.

The consistent differences in the ketosteroid excretion patterns in these 5 cases of the adrenogenital syndrome with hyperplasia were:

1. Absence of 11-hydroxyetiocholanolone.
2. Unpredictability of dehydroisoandrosterone excretion.
3. Increases in excretion of the following steroids: pregnanolone, androsterone, etiocholanolone, 11-ketoetiocholanolone, 11-hydroxyandrosterone (including 9(11)-androstenolone), pregnanetriol, and 17-hydroxypregnanolone.

DISCUSSION

In considering the significance of the altered ketosteroid excretion patterns found in these patients with congenital adrenal hyperplasia, the metabolites must be related to their precursors elaborated by the adrenal cortices. *In vivo* metabolic studies after administration of known steroids to human subjects have indicated the relationship between the urinary metabolites and their steroid precursors. These relationships have been summarized by Dorfman (17), and a simplified diagram which also incorporates the recent findings of Jailer *et al.* (1) is presented in Figure 2. In this diagram are shown only the precursors of the metabolites with which we are concerned; all of the metabolites of the precursors shown are not listed.

Our findings of increased amounts of pregnanetriol, 17-hydroxypregnanolone, pregnanolone, and etiocholanolone confirm previous evidence (1, 18–23) of increased excretion of these compounds in congenital adrenal hyperplasia and suggest that 17-hydroxyprogesterone is being secreted in increased quantities, since it can account for all of these metabolites as indicated in Figure 2. Although this does not preclude the possibility of increased secretion of other precursors of these metabolites, it bolsters the view (22) that in most of these patients the adrenocortical biosynthetic defect is such as to lead to the abnormal secretion of the postulated intermediate (24), 17-hydroxyprogesterone.

The present findings of androsterone/etiocholanolone excretion ratios greater than unity were also found by Rubin *et al.* (23) and Jailer *et al.* (1). The consistency of this ratio in patients with adrenal hyperplasia makes untenable the possibility that 17-hydroxyprogesterone is the major precursor for both of these substances, since it was shown by Jailer *et al.* (1) that the androsterone/etiocholanolone excretion ratio after administration of 17-hydroxyprogesterone was 1:5. It is thus characteristic that the human metabolic pathways that degrade 17-hydroxyprogesterone yield principally urinary 5β forms, *e.g.*, 17-hydroxypregnanolone, preg-

nanetriol and etiocholanolone. Although the increased excretion of etiocholanolone may be attributed to the increased secretion of 17-hydroxyprogesterone, the latter compound cannot account for the increased excretion of androsterone.

The other precursors of urinary androsterone may be androstenedione, testosterone, and dehydroisoandrosterone. There is no direct evidence for testosterone production in the female and its secretion in young boys

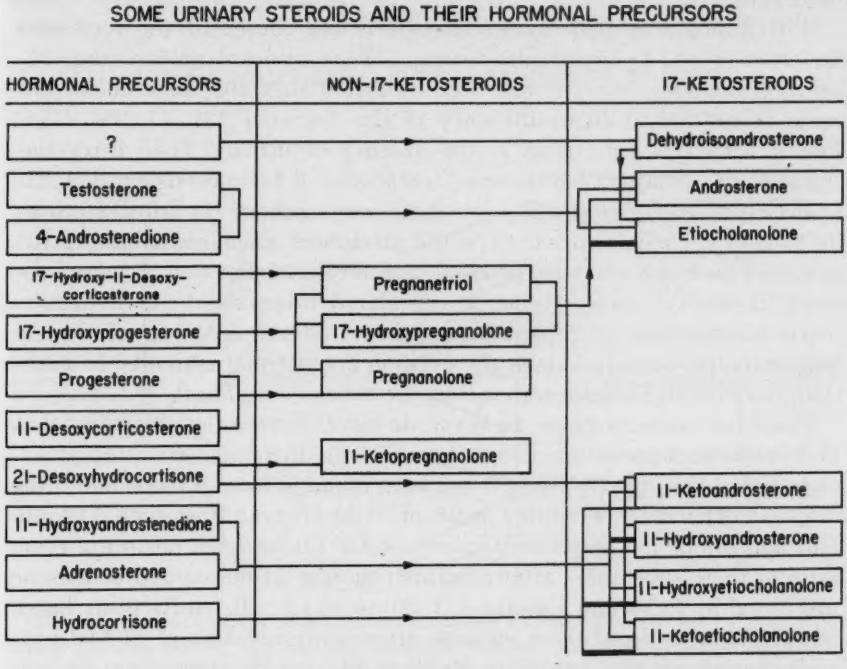

FIG. 2. Simplified diagram showing the steroid precursors of some urinary steroid metabolites. Heavy lines indicate preferential pathways.

may be considered to be negligible. Urinary androsterone is derived principally from androstenedione and dehydroisoandrosterone. Their metabolism leads to approximately equal amounts of androsterone and etiocholanolone (17), but the variability of dehydroisoandrosterone excretion makes androstenedione a more likely choice as the precursor which makes the largest and most consistent contribution to urinary androsterone titers. Androstenedione has been isolated from the adrenal cortex and has been found in human adrenal blood (25) and in adrenal perfusates (26) after administration of corticotropin.

The increased amounts of 11-oxygenated steroids in both the C_{19} and

C_{21} series which were found in these urines may be derived from the precursors shown in Figure 2. 11-Hydroxyandrosterone has been found in greatly increased quantities in cases of adrenal hyperplasia (1, 19, 20, 23) and, although 11-ketoetiocholanolone has been found in increased quantities in adrenal hyperfunction, the increases are not as striking as is the increase in 11-hydroxyandrosterone (1, 23). 11-Ketopregnanolone has been found in increased quantities in congenital adrenal hyperplasia by Jailer *et al.* (1).

It is improbable that hydrocortisone is the source of the increased excretion of 11-hydroxyandrosterone, 11-ketoetiocholanolone and 11-ketopregnanolone, since it has been indicated that the basic defect appears to be due to an insufficiency of this hormone (21, 27, 28). Consonant with this conclusion is the absence of urinary 11-hydroxyetiocholanolone, a major 17-ketosteroid metabolite of hydrocortisone (17). 21-Desoxyhydrocortisone and 11-hydroxyandrostenedione (or adrenosterone, its 11-keto analogue) appear to be the precursors which contribute to the increased excretion of 11-oxygenated ketosteroids. Jailer *et al.* (1) administered 21-desoxyhydrocortisone, a postulated intermediate in hydrocortisone biosynthesis, to 2 patients and found 5β and 5α forms of the 11-oxygenated 17-ketosteroids in the urine in about equal amounts. In addition, they found 11-ketopregnanolone in increased amounts.

These last considerations, however, do not eliminate the possibility that 11-hydroxyandrostenedione contributes to the increased excretion of 11-oxygenated ketosteroids, since it has been found in human blood and urine (25, 29). The mean excretion ratio of 11-hydroxyandrosterone/11-ketoetiocholanolone in the present cases was 2.8:1.0, and the mean excretion ratio of these substances after administration of 21-desoxyhydrocortisone was found by Jailer and associates (1, 30) to be 1.4:1.0. On the other hand, the excretion ratio of these steroids after administration of 11-hydroxyandrostenedione was found by Bradlow (31) to be approximately 5:1, indicating that both 21-desoxyhydrocortisone and 11-hydroxyandrostenedione are contributing to increased excretory titers of 11-hydroxyandrosterone and 11-ketoetiocholanolone.

The foregoing conclusions indicate that 3 androgenic substances are being secreted in increased quantities in this syndrome: androstenedione, 11-hydroxyandrostenedione, and 17-hydroxyprogesterone. The last named steroid is considered to be weakly androgenic (1) but its large increase in congenital adrenal hyperplasia (as demonstrated by the increases in its urinary metabolites) raises the possibility that it contributes to the androgenicity which leads to virilization. The concept of the overproduction of a single androgen pathognomonic of the disease is too rigid; in all probability there is hypersecretion of several androgenic steroids.

The chemical abnormalities in the adrenogenital syndrome with hyperplasia in the patients studied have been summarized in Figure 3. The deficiency in hydrocortisone secretion leads to increased amounts of circulating corticotropin and consequent adrenal hyperplasia. The hyperplastic adrenal secretes increasing quantities of the steroids which it is capable of producing, *i.e.*, C_{19} steroids as androstenedione and 11-hydroxyandrostenedione and also C_{21} biosynthetic intermediates such as 21-desoxyhydrocortisone and 17-hydroxyprogesterone. Some of these steroids, being androgenic, may well produce the virilization. Their characteristic metab-

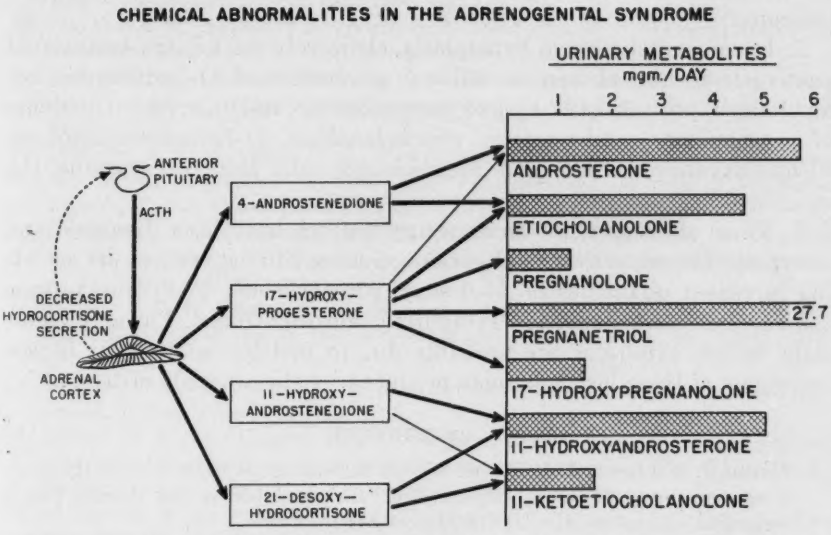

FIG. 3. Summary of the chemical abnormalities in congenital adrenal hyperplasia. Heavy lines on the left side indicate increased secretion and heavy lines on the right, preferential metabolic pathways leading to the mean steroid excretion pattern of female pseudohermaphrodites.

olism leads to the altered urinary ketosteroid patterns which have been shown. Whereas the metabolism of the C_{21} intermediates leads to more singular excretion products, such as pregnanetriol and 17-hydroxypregnanolone, the metabolic alterations of the C_{19} steroids lead to excretory products common to the other steroid hormones, obscuring their role in the production of the virilization.

A metabolic variant of the patient with adrenal hyperplasia whose urinary ketosteroids do not conform to the foregoing pattern has recently been described by Bongiovanni and Eberlein (2, 37). Such cases are complicated by hypertension and indicate that the block in the biosyn-

thesis of hydrocortisone occurs at a step which leads to a preponderance of Reichstein's compound S and its metabolites in blood and urine. These unusual cases indicate that the enzymatic defect may occur at one or more points in the biosynthetic pathways.

SUMMARY

1. The urinary ketosteroid excretion patterns of 4 normal subjects and 5 patients with congenital adrenal hyperplasia have been investigated. The method involves a three-step hydrolytic process, ketosteroid separation by alumina chromatography, and identification by means of infrared spectroscopy.

2. In congenital adrenal hyperplasia, changes in the urinary ketosteroid pattern from normal were as follows: a) absence of 11-hydroxyetiocholanolone; b) presence of 17-hydroxypregnanolone; and c) increased amounts of pregnanolone, androsterone, etiocholanolone, 11-ketoetiocholanolone, 11-hydroxyandrosterone, and pregnanetriol—the last two showing the most striking increases.

3. These alterations in the excretory pattern have been discussed and interpreted to mean that the hyperfunctioning adrenal cortices are secreting increased quantities of 21-desoxyhydrocortisone, 17-hydroxyprogesterone, androstenedione, and 11-hydroxyandrostenedione. The androgenic signs in this syndrome are probably due to multiple androgenic hypersecretions of these last 3 steroids produced by the adrenal cortices.

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METABOLISM OF FREE AND CONJUGATED 17-HYDROXYCORTICOSTEROIDS IN NORMAL SUBJECTS*

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INTRODUCTION

CONSIDERABLE information concerning the metabolism of the 17-hydroxycorticosteroids (17-OHCS) has accumulated as a result of the introduction of methods for measuring the free material in various body fluids (1). However, a sizable fraction of the total 17-OHCS in the plasma and the major portion of the 17-OHCS in the urine are not measured by these methods because the corticoids are esterified with glucuronic acid. The recent development of methods for the estimation of 17-OHCS conjugated with glucuronic acid (2) has made possible an extension of our knowledge of 17-OHCS metabolism.

Techniques for the measurement of free and conjugated 17-OHCS have been employed in our laboratory to study 17-OHCS metabolism in a variety of clinical situations. The results of such studies in normal human subjects are presented in this communication. These observations will be compared in subsequent papers with observations made in patients with thyroid (3), liver (4) and kidney (5) disease.

MATERIALS AND METHODS

Subjects, dosage and blood sampling

The 58 "normal" subjects were hospital personnel or ambulatory patients at the end of hospitalization for psychoneurosis, peptic ulcer or infections. In each instance endocrine, liver and renal disease were excluded by clinical and laboratory evaluation. Endogenous creatinine clearance and bromsulfalein retention at forty-five minutes after an intravenous dose of 5 mg. per Kg. were normal in each subject.

In 10 of these subjects (mean age, 30 years), serial samples of blood were drawn during a 24-hour period to assess the diurnal variation of the levels of the free and conjugated 17-OHCS in the plasma (Fig. 1).

To 14 subjects (mean age, 34 years), a standard infusion of cortisol was administered in accordance with the previously published protocol (6). Blood samples for the estimation of free and conjugated 17-OHCS levels in the plasma were drawn at zero, one-half,

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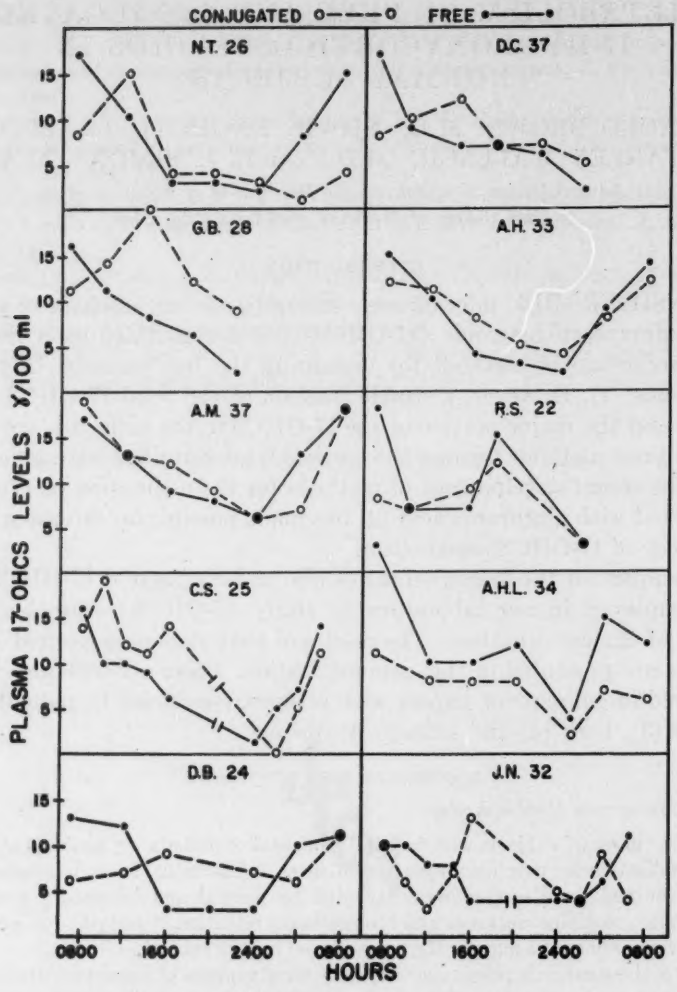

FIG. 1. Diurnal variation of the free and conjugated 17-OHCS levels in the plasma of 10 normal subjects.

one, two, four and six hours after the beginning of the infusion, which lasted thirty minutes. Three of the studies were extended to ten hours.

To 18 subjects (mean age, 41 years), a dose of 25 International Units of corticotropin (ACTH) was administered intravenously over a six-hour period and the plasma levels of free and conjugated 17-OHCS were measured at zero, two, four and six hours after the beginning of the infusion (7).

To 9 subjects, an intravenous infusion of tetrahydrocortisone was given over a thirty-minute period (6); 6 received tetrahydrocortisol and 7 received dihydrocortisone in a

similar manner. Serial samples of blood for the estimation of free and conjugated 17-OHCS levels in the plasma were drawn over a six-hour period.

Three subjects received an infusion of tetrahydrocortisone intravenously over a five-minute period. Two received an infusion of dihydrocortisone, and 2 an infusion of tetrahydrocortisol—also within five minutes. This was accomplished by elevation of the infusion bottle and vasodilatation of the recipient arm by means of heat. Serial samples of blood were drawn over a two-hour period.

Chemical methods

The level of free 17-OHCS in the plasma was measured by the technique of Eik-Nes, Nelson and Samuels (8), and that of conjugated 17-OHCS by a modification of the technique of Bongiovanni (2). The details of the modified method are as follows:

Determination of conjugated 17-OHCS in plasma. A 10-ml. sample of plasma was pipetted into a 50-ml. centrifuge tube and extracted with chloroform in the usual way for determination of *free* 17-OHCS (8). It was then stored in the 50-ml. tube in the frozen state for later analysis for *conjugated 17-OHCS.*

The plasma was allowed to thaw at room temperature and was mixed with 8 ml. of 0.2 *M* sodium acetate-acetic acid buffer (pH 4.5). The mixture was centrifuged and any remaining chloroform from the free 17-OHCS extraction was removed with a plasma lifter. This instrument was used also to break up the protein precipitate and was then rinsed with the buffer. A 2-ml. portion of "Ketodase" (Warner-Chilcott) containing 5,000 units of β-glucuronidase per ml. was added to the 50-ml. tube and the mixture was incubated at 37° C. for twenty-four hours.

Cellophane tubing[1] for dialysis bags was moistened and cut into 10-inch lengths. These were boiled for four hours in four changes of distilled water before use. One end of the tubing was knotted and the hydrolyzed plasma was poured into the other end with the aid of a funnel. The incubation tube was rinsed twice with 2 ml. of distilled water and the rinses were added to the contents of the cellophane bag. The open end of the tubing was knotted. The knot was trimmed with scissors and rinsed thoroughly with a stream of distilled water to remove all traces of protein. The filled dialysis bag was placed in a 100-ml. graduated cylinder containing 50 ml. of distilled water. Dialysis was carried out for forty-eight hours at room temperature in the dark. The water was changed at twenty-four and at thirty-six hours and the pooled dialysate was kept in a stoppered 250-ml. round-bottom centrifuge tube in the refrigerator.

After the addition of 50 ml. of chloroform (freshly distilled over anhydrous potassium carbonate) to the pooled dialysate, vigorous mixing was carried out with a footed glass rod. No centrifugation was necessary if all traces of protein had been removed from the external surface of the dialysis bag. The chloroform extract was removed with a plasma lifter and filtered through anhydrous sodium sulfate into a 250-ml. Erlenmeyer flask. The extraction procedure was repeated three times and the pooled extract was evaporated to dryness in a water bath at 37° C. under a gentle stream of air.

Subsequent column chromatography and color development were carried out in accordance with the technique of Eik-Nes, Nelson and Samuels (8), except that results were reported in terms of a cortisone standard.

Mean recoveries of free tetrahydrocortisone added to plasma which had been extracted with chloroform ranged from 85 per cent to 95 per cent with this technique. Increased amounts of enzyme, longer incubation, or additional extractions of the

[1] "Nojax" casings, size #24, made by the Visking Corporation, Chicago, Illinois.

dialysate failed to produce additional chromogen. Extraction of the incubation mixture after dialysis failed to uncover Porter-Silber chromogens in excess of background. Unfortunately, pure tetrahydrocortisone glucuronide has not been available to us for recovery studies.

RESULTS

8 a.m. levels of free and conjugated 17-OHCS

The mean level of free 17-OHCS (with the standard error of the mean) was 12.5 ± 0.5 μg. per 100 ml. (58 subjects), and of the conjugated 17-OHCS 10.4 ± 0.6 μg. per 100 ml. (51 subjects).

Diurnal variation

The diurnal variation of the levels of free and conjugated 17-OHCS in the plasma of 10 subjects is shown in Figure 1. In 8 of the 10, the level of

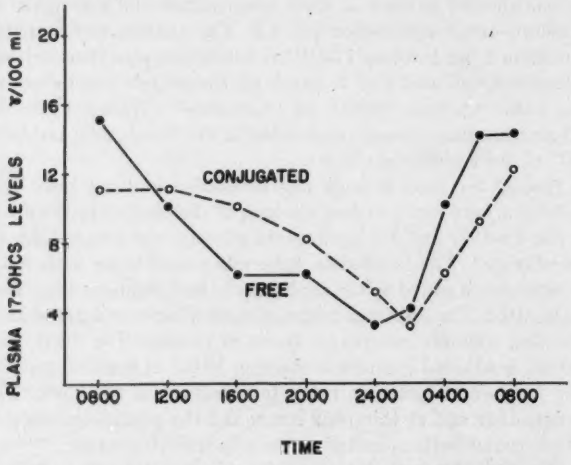

FIG. 2. Curves of diurnal variations in the levels of free and conjugated 17-OHCS in plasma, drawn from the mean blood levels of the subjects in Figure 1 after extrapolation to uniform times.

free 17-OHCS was highest between 6 a.m. and 8 a.m. and fell until a period between 12 midnight and 4 a.m., at which time there was a sharp rise to the morning peak. The levels of conjugated 17-OHCS followed the same pattern, but lagged behind by a period of two to four hours. Because of the variation in the individual cases, these relationships are best illustrated in Figure 2, in which the mean extrapolated levels at uniform times are plotted.

In 2 subjects, R.S. and J.N., there was a deviation from the usual pattern at one point late in the day.

Plasma levels of free and conjugated 17-OHCS after cortisol infusion

The mean levels of free and conjugated 17-OHCS in plasma following the cortisol infusion are plotted in Figure 3. The mean level of free 17-OHCS in plasma (with the standard error of the mean) was 12 ± 0.7 μg. per 100 ml. at zero hour; 119 ± 9.3 at half an hour; 83 ± 2.9 at one hour; 57 ± 2.3 at two hours; 28 ± 1.4 at four hours; 13 ± 1.0 at six hours; and 3.7 ± 1.9 at ten hours. When the logarithms of the plasma levels of free

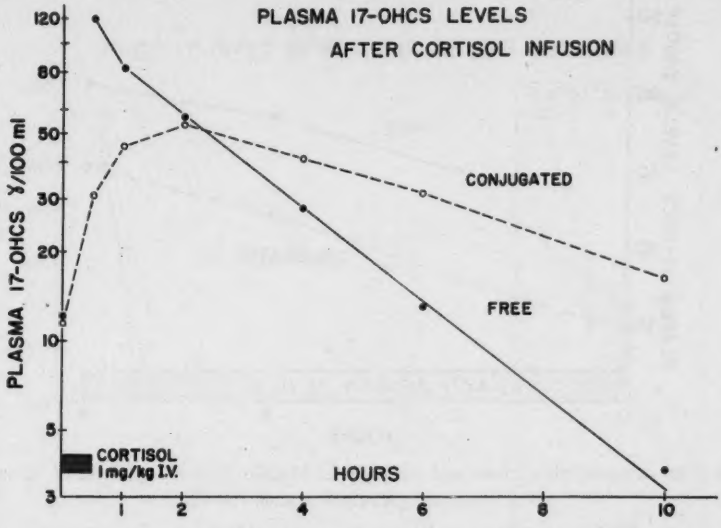

FIG. 3. The mean levels of free and conjugated 17-OHCS in the plasma of 14 normal subjects after the infusion of cortisol. In the calculation of the mean level of free 17-OHCS, 11 subjects of the previous study (6) were added to the present group (total, 25 subjects).

17-OHCS were plotted against time, the points fell on a straight line after the one-hour value. The mean slope of the line (with the standard error of the mean) was -0.162 ± 0.007 and the mean half-life of the administered cortisol was 112 ± 5 minutes.

The mean level of conjugated 17-OHCS in plasma (with the standard error of the mean) was 12 ± 1.6 μg. per 100 ml. at zero hours; 32 ± 3.3 at half an hour; 45 ± 3.8 at one hour; 53 ± 3.5 at two hours; 41 ± 3.0 at four hours; 31 ± 2.0 at six hours; and 16 ± 4.6 at ten hours. The plasma levels of conjugated 17-OHCS rose to a peak of 53 μg. per 100 ml. at two hours after the beginning of the infusion and then declined more slowly than did the free levels. The disappearance of the conjugated 17-OHCS appeared to follow a straight line when plotted logarithmically against time.

Plasma levels of free and conjugated 17-OHCS following stimulation with ACTH

The mean levels of free and conjugated 17-OHCS in plasma after administration of ACTH are shown in Figure 4. The mean free 17-OHCS level (with the standard error of the mean) rose from 12 ± 1.1 μg. per 100 ml. to 41 ± 3.2 μg. per 100 ml. The mean level of conjugated 17-OHCS

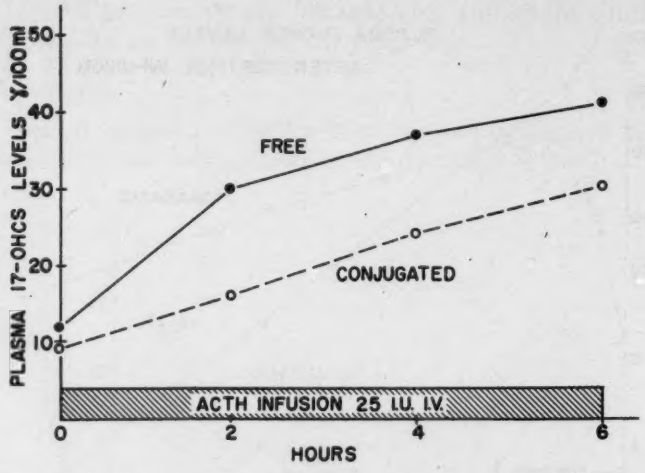

FIG. 4. The mean levels of free and conjugated 17-OHCS in the plasma of 18 normal subjects after stimulation with ACTH.

in the plasma rose from 9 ± 1.1 μg. per 100 ml. to 30 ± 2.2 μg. per 100 ml. There appeared to be a linear increase in the levels of conjugated 17-OHCS in the plasma during the period of study in all but 3 subjects.

Plasma levels of free and conjugated 17-OHCS after infusion of tetrahydrocortisone (Tetra E), tetrahydrocortisol (Tetra F) and dihydrocortisone (Di E)

The mean levels are plotted in Figures 5 and 6. Immediately after each infusion, the free 17-OHCS disappeared from the plasma at a rate which was much more rapid than that of cortisol. When the logarithms of the free levels were plotted as a function of time, there was a suggestion of a straight-line relationship for one to two hours after the thirty-minute and after the rapid five-minute infusions (Fig. 5). During this interval there were no striking differences between the rates of removal of the 3 substances. Following the initial phase, the levels of free 17-OHCS declined at a progressively slower rate for a short period of one-half to one hour. The removal rates of 17-OHCS after the infusions of Tetra E and Di E

were similar at this time and were faster than the removal rate after administration of Tetra F. Thereafter, the levels of free 17-OHCS appeared to fall in an exponential fashion for the remainder of the six-hour study. During this final period, the free levels after the infusion of Tetra F fell more rapidly than those after the infusions of the other two materials. Control levels were achieved six hours after the beginning of the infusion of each substance.

The levels of conjugated 17-OHCS after the infusion of these substances

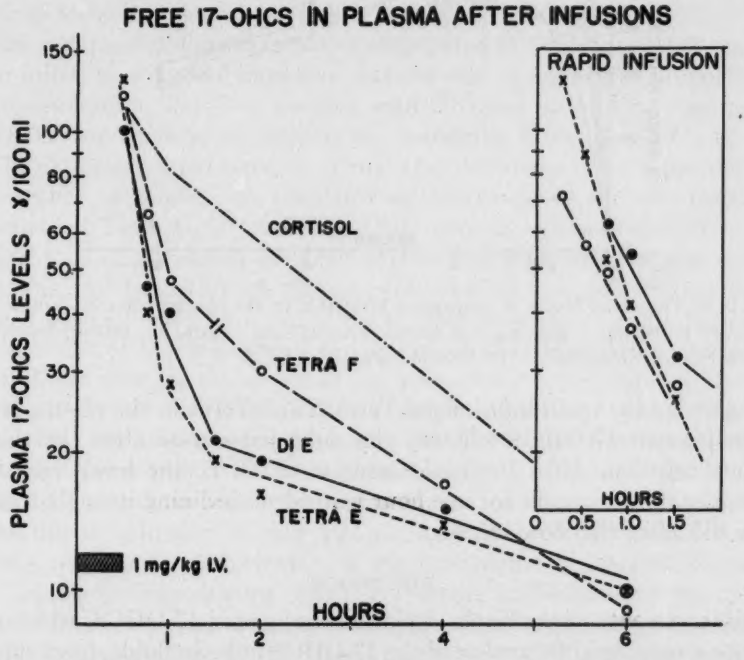

FIG. 5. The mean levels of *free* 17-OCHS in the plasma after 30-minute and 5-minute infusions (1 mg./Kg.) of tetrahydrocortisol (Tetra F), tetrahydrocortisone (Tetra E), dihydrocortisone (Di E) and cortisol.

rose higher and much more rapidly than after the infusion of cortisol. Immediately after the thirty-minute infusion of Di E and of Tetra E, the levels were at a peak; they subsequently declined logarithmically with time. In contrast, the levels of conjugated 17-OHCS after administration of Tetra F did not reach a maximum until one hour after the beginning of the infusion. The levels in the samples drawn at two to six hours also declined exponentially.

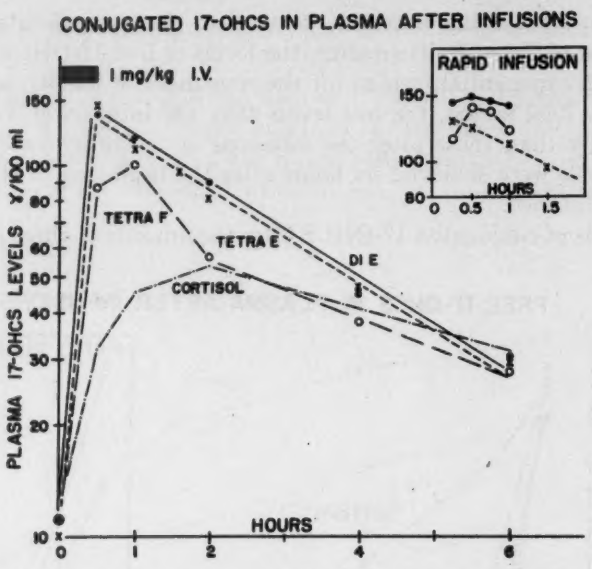

FIG. 6. The mean levels of *conjugated* 17-OHCS in the plasma after 30-minute and 5-minute infusions (1 mg./Kg.) of tetrahydrocortisone (Tetra E), tetrahydrocortisol (Tetra F), dihydrocortisone (Di E) and cortisol.

Following the rapid infusions of Tetra E and Tetra F, the plasma levels of conjugated 17-OHCS followed the same pattern as after the thirty-minute infusion. After the rapid infusion of Di E, the levels remained approximately constant for one hour instead of declining immediately, as they did after the slow infusion.

DISCUSSION

Measurements of the levels of plasma conjugated 17-OHCS, which constitute a considerable portion of the 17-OHCS in body fluids, have supplemented our knowledge of corticosteroid metabolism. In the situations studied, the catabolism of cortisol resulted in the formation of conjugated 17-OHCS. The levels of the free and conjugated 17-OHCS at 8 a.m. were approximately equal. After an infusion of cortisol, the level of free 17-OHCS declined rapidly, whereas the level of conjugated 17-OHCS rose over a two-hour period to a peak value which was about equal to the value of free 17-OHCS at this time. It is probable that the rate of formation of conjugated 17-OHCS is governed by the rate of conversion of cortisol to reduced corticosteroids such as Tetra E and Tetra F, and that these are subsequently combined very rapidly with glucuronic acid (9). The rate of reduction of circulating free 17-OHCS is controlled by enzymatic processes

in the liver (10). The conjugated 17-OHCS are removed from the plasma by glomerular filtration (5, 11, 12). Thus, the actual levels of conjugated 17-OHCS in the plasma depend upon the rate of formation from cortisol and the rate of excretion by the kidney.

It is of interest that the levels of conjugated 17-OHCS in the plasma exhibited a pattern of diurnal variation similar to, but two to four hours later than, those of free 17-OHCS (13). During the six-hour period of ACTH stimulation, the rise in the levels of the conjugated 17-OHCS again lagged behind those of the free material. The time-lag probably represents the interval for conversion of cortisol to conjugated 17-OHCS.

The exponential disappearance of conjugated 17-OHCS from the plasma after infusion of cortisol indicates that the rate of removal is proportional to concentration. This is in keeping with the observation that conjugated 17-OHCS are excreted principally by glomerular filtration (5, 11, 12).

The extremely rapid removal of free 17-OHCS from the plasma and the appearance of conjugated 17-OHCS in the plasma of subjects receiving infusions of Tetra E, Tetra F and Di E were in striking contrast to the results following infusion of cortisol. The changing slope of the curves depicting the logarithm of the concentration of free 17-OHCS plotted against time after the infusions of the cortisol metabolites is interpreted to be the result of the endogenous production of 17-OHCS. After infusion of cortisol, the endogenous output of the adrenal cortex is inhibited, so that the levels of free 17-OHCS exhibit a simple logarithmic decay (Fig. 5). Since the rates of conjugation of all 3 cortisol metabolites were similar and much more rapid than that following administration of cortisol, it seems likely that, in man, the limiting reaction of cortisol metabolism is the initial reduction of ring A of the cyclopentanohydrophenanthrene nucleus, resulting in the formation of dihydrogenated derivatives. Tomkins and Isselbacher have shown that this reaction is the limiting one in the isolated enzyme system of rat liver (10). It would have been instructive to study the metabolism of dihydrocortisol along with the other cortisol metabolites, but this compound was not available for clinical studies at the time.

From our limited data there is also some suggestion that, after the infusion of Tetra F, conjugated 17-OHCS were not formed as readily as when the other two reduced 17-OHCS were infused. Also, the free material did not disappear from the plasma as quickly as when Tetra E or Di E was administered. This may indicate that at least a portion of the Tetra F was converted to other reduced 17-OHCS prior to conjugation. Tetra F has been identified along with Tetra E in the conjugated portion of 17-OHCS in human plasma (5, 14).

There is no evidence that these reactions have anything to do with the

physiologic action of the hormones of the adrenal cortex. It is more probable that they represent mechanisms for removing the metabolically active steroids (15) which are produced in excess by the organism. At any rate the measurement of the levels of both conjugated and free 17-OHCS serves to clarify further the metabolism of the hormones of the adrenal cortex.

SUMMARY

1. The levels of free and conjugated 17-OHCS in the plasma of normal subjects at various times of the day, and after infusions of cortisol, corticotropin, tetrahydrocortisone (Tetra E), tetrahydrocortisol (Tetra F) and dihydrocortisone (Di E) have been measured.

2. After infusion of cortisol or corticotropin, the rise in plasma levels of conjugated 17-OHCS lagged behind the rise in the levels of the free substances by two to four hours. After infusions of hydrogenated derivatives of cortisol, the conjugation reaction was very rapid.

3. There was a diurnal variation in the levels of both free and conjugated 17-OHCS in the plasma. Here, again, the changes in the levels of conjugated 17-OHCS lagged behind the changes in the levels of free 17-OHCS by two to four hours.

4. From the time-concentration relationships of the free and conjugated 17-OHCS levels in the plasma after the intravenous administration of cortisol metabolites, additional support can be offered for the hypothesis that cortisol is reduced to tetrahydro compounds and that these are subsequently rapidly conjugated with glucuronic acid and excreted by glomerular filtration.

Acknowledgments

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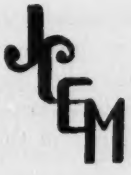

GRANULOMATOUS THYROIDITIS (DE QUERVAIN'S THYROIDITIS)*

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GRANULOMATOUS thyroiditis is a distinct clinicopathologic entity, apparently a true nonsuppurative inflammation of the thyroid gland, that can be distinguished from the rare invasive fibrous thyroiditis (Riedel's struma) or the more common struma lymphomatosa. Since its first description by de Quervain in 1904 (1), the disease has been reported under many synonyms. Terms that call attention to the histologic features of the lesion include "pseudotuberculous thyroiditis," "granulomatous thyroiditis," "giant-cell thyroiditis" or "struma fibrosa, giant-cell variant." The clinical findings have given origin to such names as "acute and subacute nonsuppurative thyroiditis," "acute noninfectious thyroiditis," "acute diffuse thyroiditis" and "subacute thyroiditis." The latter term is commonly used. However, since there appears to be one basic pathologic process with extreme variation in severity of symptoms, and since many cases undoubtedly occur in which subjective symptoms are absent, it was thought best to use the name that calls attention to the distinctive pathologic findings, namely "granulomatous thyroiditis."

The true incidence of this disease, and its incidence relative to other types of thyroiditis, are not readily discernible from study of the literature (2). In 1936, de Quervain and Giordanengo (3) collected reports of 54 cases appearing in the literature since de Quervain's original description of the disease. Some of the more recent articles have emphasized that granulomatous thyroiditis is a distinct clinicopathologic entity (4–8), but several cases have been reported under other designations (9–12). Fraser and Harrison (13) stated that the disease is uncommon in England.

A review of our cases was undertaken to compare the incidence, course and prognosis with those of Riedel's struma and to emphasize the wide variation in clinical symptomatology in the disease.

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MATERIALS AND METHODS

All cases of granulomatous thyroiditis seen at the Mayo Clinic during a 27-year period (1930 through 1956) in which thyroidal tissue was available were selected for study. The method of case finding has been outlined in our previously published paper on Riedel's struma (14). In brief, the findings in all cases originally diagnosed as "thyroiditis" of any type, as well as in all cases of adenomatous goiter in which thyroiditis was associated, were reviewed. Cases of Graves' disease were re-examined only if a pronounced degree of thyroiditis was mentioned in the original report. All cases of "tuberculosis" of the thyroid also were reviewed. All gross specimens were available for study. These were examined carefully to determine the gross extent of the pathologic process and to see whether the uninvolved thyroidal tissue was normal. Multiple new sections were obtained from the zones of thyroiditis and from normal tissue, when present. The presence or absence of adherent muscle or other soft tissue was noted.

Examples of invasive fibrous thyroiditis (Riedel's struma) and typical examples of struma lymphomatosa, including the more fibrotic variety, were readily diagnosed in this manner. A large number of cases of what provisionally has been called "chronic lymphocytic thyroiditis" also were noted.

A group of cases remained in which inflammation, apparent loss of follicular epithelium and associated evidence of breakdown of colloid by macrophages and giant cells were prominent features. All these cases were classified as granulomatous thyroiditis. In addition; a few specimens showed focal scars in thyroidal tissue without evidence of active inflammation. These cases were excluded from the study, although most of them were presumed to represent the end-stage of a previous attack of granulomatous thyroiditis. Various stages of the disease were represented in this series; in order to demonstrate the terminal or residual pathologic changes, 2 cases were included in which typical clinical and laboratory findings of granulomatous thyroiditis were noted at a previous visit to the clinic and thyroid tissue was obtained from the involved lobes several years later.

It is recognized that a minority of patients who have granulomatous thyroiditis undergo biopsy or surgical resection of the thyroid. Consequently, in order to determine the total incidence of the disease and what proportion of patients are treated surgically in our practice, a survey was made of the records of all clinically diagnosed cases seen and treated at the clinic during the last five years of this study (1952 through 1956). For this period, the records in all cases of acute thyroiditis, acute diffuse thyroiditis, subacute thyroiditis or granulomatous thyroiditis were reviewed.

DEFINITION OF GRANULOMATOUS THYROIDITIS

The definition of granulomatous thyroiditis is essentially a pathologic one. All cases included in this study were selected on the basis of the results of pathologic examination of thyroidal tissue, without regard to the clinical status of the patient. The combined gross and microscopic features are sufficiently distinctive for the diagnosis of this disorder and are unlike those in any other disease entity involving the thyroid.

The microscopic findings in the first few days after onset of the disease are rather poorly documented, because operation or needle biopsy is rarely performed at this time. The typical patient is seen from two weeks to three months after the apparent onset, and the histologic findings at this

time are fully diagnostic. At this stage, the histologic picture points to an inflammatory process, with destruction of follicular epithelium as a prominent feature. The preformed colloid becomes surrounded by a zone of inflammatory or phagocytic cells; including polymorphonuclear leukocytes, macrophages and foreign-body giant cells. Associated with these changes is interstitial infiltration by inflammatory cells, with variable amounts of fibrosis, depending on the stage of the disease. The over-all picture is one of progressive destruction of thyroidal parenchyma, sometimes apparently fanning out from a single focus or sometimes diffusely involving the gland. However, this destruction is rarely complete and islands of small regenerating thyroidal follicles usually can be seen in the older zones after the acute stage has passed.

Thus, the early stages of the disease generally are characterized by active inflammation, destruction of follicles and a tuberculoid reaction, and the later stages usually by healing and fibrosis. Frequently, however, both phases are seen in the same gland; the active zone spreads out to encroach on apparently normal parenchyma, leaving behind a central scarred region. Pathologic data in cases of older subsiding disease are fairly abundant and reasonably diagnostic also, although much less of the inflammatory granulomatous process is apparent at this time. Clinically, the process subsides completely in the usual case and no residual enlargement is palpable. Consequently, pathologic examination of thyroidal tissue at this terminal stage is entirely fortuitous.

RESULTS AND COMMENTS

A detailed study of only the cases of granulomatous thyroiditis proved pathologically is included in this report. The aforementioned 5-year review of clinical cases was conducted to obtain data on the over-all incidence of this disease.

Incidence

Granulomatous thyroiditis in our experience is a fairly common form of thyroiditis, although pathologic proof of the disease is obtained somewhat infrequently. The disease is much more common than invasive fibrous thyroiditis (Riedel's struma) but less frequent than struma lymphomatosa. A total of 108 cases of granulomatous thyroiditis were found during the 27-year period covered by this study. During approximately the same period, 20 cases of Riedel's struma were found. This obviously does not represent a valid comparison of the two diseases, since all patients with Riedel's struma theoretically are treated surgically, whereas a minority of patients who have granulomatous thyroiditis are so treated.

Results of the 5-year survey of all our cases of granulomatous thyroiditis, including those in our series proved pathologically and those diagnosed clinically only, are shown in Table 1. During this 5-year period 162 cases were observed; either biopsy or surgical resection was performed in 37 (23 per cent). Sixteen of these 37 patients, or 10 per cent of the total number seen, were systemically asymptomatic and the only complaint

TABLE 1. INCIDENCE OF GRANULOMATOUS THYROIDITIS AT THE MAYO CLINIC DURING A 5-YEAR PERIOD

Year	Patients	Diagnosis		Severity of general symptoms in surgical patients		
		Clinical	Surgical*	Asymptomatic (lump in neck only)	Mild to moderate	Severe
1952	33	24	9	2	4	3
1953	30	24	6	4	2	0
1954	39	28	11	4	6	1
1955	32	23	9	4	5	0
1956	28	26	2	2	0	0
Total	162	125	37	16	17	4

* Needle or surgical biopsy or surgical resection.

referable to the neck was that of a lump; whereas the remainder of the patients had mild, moderate or severe subjective symptoms of granulomatous thyroiditis. All the 125 cases diagnosed clinically obviously had typical symptoms and laboratory findings of the disease.

During this same 5-year period, 3 patients with Riedel's struma were seen, and approximately 1,250 patients with Graves' disease were seen and treated either surgically or with radioiodine. Therefore, granulomatous thyroiditis in our current practice is approximately one eighth as common as Graves' disease but at least fifty times more common than Riedel's struma.

Age and sex

The 108 patients in whom granulomatous thyroiditis was pathologically proved included 84 women and 24 men, the ratio of women over men being 3.5:1. The ages ranged from 29 to 73 years, with an average of 49.4 years.

Previous goiter

Twelve patients gave histories of goiters for periods up to thirty-two years before the onset of granulomatous thyroiditis. Pathologic examina-

tion in 8 of these revealed either a single adenoma (7 cases) or nontoxic adenomatous goiter (1 case); in the remaining 4 cases there were no changes to account for the previous goiter.

The duration of a lump in the neck associated with granulomatous thyroiditis was known in 70 cases; in all, the duration was relatively short. Half the patients had noted the lump for one month or less, whereas 30 per cent had noted it for one or two months and the remainder for three or four months. The duration of the swelling in the thyroidal region coincided well with the duration of subjective symptoms, when such were present.

Findings in "asymptomatic" patients

A surprising feature of this study of granulomatous thyroiditis, as defined here, and one that has been little emphasized heretofore, was the occurrence of the disease in a large number of patients as an asymptomatic process as far as the usual symptoms of thyroiditis are concerned, *i.e.*, these patients were completely free of the characteristic subjective symptoms associated with thyroiditis, such as pain, tenderness and dysphagia. There were 37 such "asymptomatic" cases in this series of 108 proved cases, or 34 per cent of the total.

These 37 patients were divided into four groups: 1) those complaining of a lump in the neck of short duration, 2) those complaining of a lump in the neck of longer duration, 3) those in whom a goiter was first detected on general physical examination, and 4) those presenting problems of Graves' disease.

Lump in neck of short duration (15 patients). A lump in the neck had been present in 12 patients for periods varying from two weeks to two months, and 3 patients had noted swelling for three to five months. The most frequent preoperative diagnosis in this group was adenomatous goiter without hyperthyroidism, but the presence of carcinoma was strongly suspected in some instances, as illustrated in the case of a 72-year-old man who complained of a painless firm mass in the right side of the neck of one month's duration. Examination revealed a firm, fixed, malignant-feeling, 2.5-cm. mass in the right upper pole of the thyroid. The patient was euthyroid. The erythrocytic sedimentation rate was 27 mm. during the first hour (Westergren method). At operation, the right lobe of the thyroid was enlarged, hard and adherent to the ribbon muscles and adjacent structures. The surgeon suspected the presence of carcinoma. The left lobe was normal. Pathologic examination of the resected lobe showed granulomatous thyroiditis involving approximately 80 per cent of the lobe (Fig. 1*a* and *b*). There was a pronounced typical granulomatous reaction with numerous micro-abscesses.

The palpable mass in each of these 15 patients was composed entirely

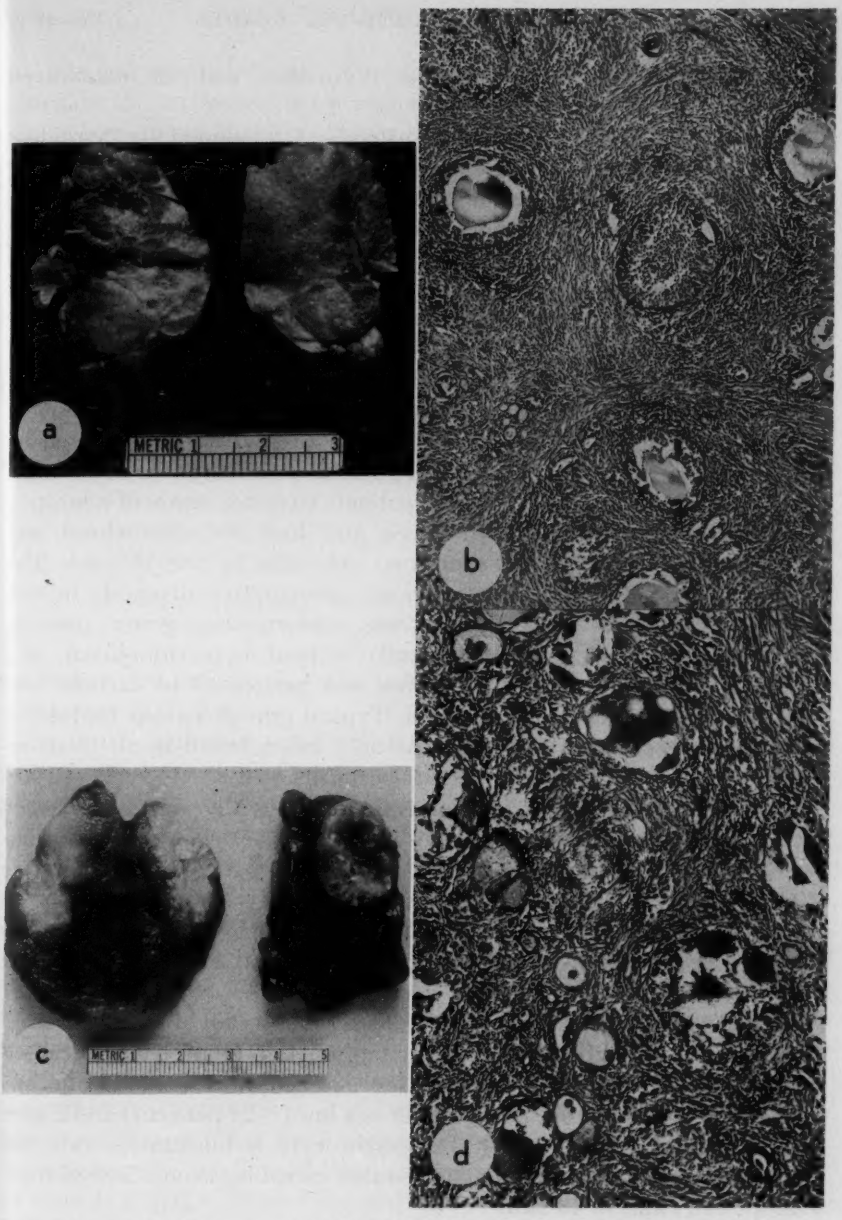

FIG. 1. **a.** Surgically resected right lobe in a 72-year-old man. **b.** Same case, showing typical active granulomatous thyroiditis with micro-abscesses (hematoxylin and eosin; ×60). **c.** Surgically resected thyroid in another case, showing a partially encapsulated grade-1 papillary and follicular 2.5-cm. adenocarcinoma in the right lobe, with clinically unsuspected focal granulomatous thyroiditis in the left lobe. **d.** Appearance of zone of thyroiditis (hematoxylin and eosin; ×120).

of tissue affected by granulomatous thyroiditis, and the uninvolved thyroidal tissue was normal.

Lump in neck of longer duration (4 patients). Granulomatous thyroiditis was discovered incidentally in 4 patients operated on for a palpable mass in the thyroid of longer duration than five months. In 1 of these, a papillary and follicular 2.5-cm. carcinoma was present in the right lobe, and a clinically unsuspected zone of granulomatous thyroiditis measuring 3 by 1.5 by 1.5 cm. was noted in the left (Fig. 1*c* and *d*). A zone of granulomatous thyroiditis was adjacent to an adenoma in the other 3 patients (Fig. 2).

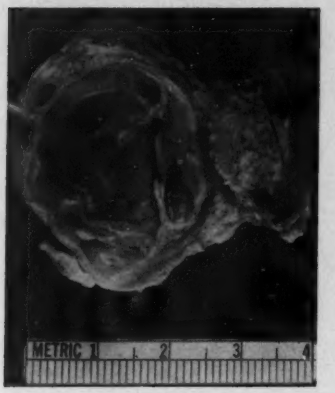

FIG. 2. Granulomatous thyroiditis as an incidental finding, with a small lesion adjacent to a cystic adenoma.

Goiter found on examination (14 patients). Masses in the thyroid were discovered on general physical examination in 14 patients. These patients were not aware of a lump in the neck and had not experienced any symptoms referable to the thyroid. The commonest preoperative diagnosis in this group was adenomatous goiter (usually unilateral) without hyperthyroidism, and operation was performed to exclude carcinoma. Typical granulomatous thyroiditis was the only lesion found in all these resected specimens.

Graves' disease (4 patients). Focal granulomatous thyroiditis was found in 2 patients believed to have Graves' disease on both clinical and pathologic grounds. The clinical picture in 2 additional patients was suggestive of Graves' disease, but pathologic examination revealed diffuse granulomatous thyroiditis without any definite microscopic evidence of hypertrophy. Lugol's solution had been given preoperatively for ten days in both these cases.

Laboratory aspects. Excluding the 4 patients suspected of having Graves' disease, 27 of the patients in this "asymptomatic" group had determinations of the basal metabolic rate. Of these, 24 had rates within our normal range (−15 to +15 per cent). One rate was low (−24 per cent) and 2 were high (+17 and +37 per cent). The erythrocytic sedimentation rate was measured in 14 of the 37 patients; the rates varied between 6 and 86 mm., with a mean value of 43 mm.

Findings in symptomatic patients

The remaining 71 of the 108 patients in this series had symptoms associated with the thyroiditis. These symptoms were mild to moderate in 64

patients and were severe in 7. The complaints most commonly mentioned were pain and tenderness in the region of the thyroid, pain referred to the ears or jaws, fever, malaise, dysphagia, and symptoms of recent pharyngitis or an upper respiratory infection (Table 2).

The basal metabolic rate was measured in 65 of these 71 patients. The mean rate was +7 per cent, with a variation from −12 to +47 per cent. The rate was more than +15 per cent in 17 patients. The sedimentation

TABLE 2. FREQUENCY OF SYMPTOMS IN 71 SYMPTOMATIC PATIENTS WITH GRANULOMATOUS THYROIDITIS

Symptoms	No. of patients
Tenderness	66
Pain	50
Dysphagia	27
Referred pain	23
Malaise	21
Fever	17
Recent pharyngitis	16

rate was determined in 45 of the patients; it varied from 8 to 124 mm., with a mean of 63.5 mm.

The usual finding in tests utilizing radioiodine tracers performed on patients who have clinical acute or subacute diffuse thyroiditis (granulomatous thyroiditis) is a virtual absence of collection of radioiodine by the thyroid (7, 15). However, of the 13 patients in this series who had I^{131} studies, 5 had a normal 24-hour thyroidal uptake of I^{131} (12 to 42 per cent), 7 had a low uptake (1 to 9 per cent), and 1 had an increased uptake (79 per cent); this last patient had Graves' disease in addition to granulomatous thyroiditis. Gammagraphic studies were carried out on 3 of these patients (all of whom had a normal uptake of I^{131}), and these showed good collection of I^{131} in the unaffected or slightly affected lobe but poor or no collection in the involved lobe. Pathologic examination in 12 of these 13 patients, excluding the 1 with Graves' disease, revealed unilateral (focal) granulomatous thyroiditis in 5 instances and bilateral disease in 7. The 24-hour uptakes of I^{131} in the 5 unilateral lesions were 4, 7, 12, 23 and 25 per cent, respectively. The 24-hour uptakes in the 7 cases of bilateral disease were 1, 1, 2, 6, 9, 11 and 42 per cent, respectively. In the patient who had bilateral disease and a 42 per cent uptake, the lesions were relatively inactive and apparently in the healing phase.

In contrast to these findings were those in the 53 patients who were given tracer doses of I^{131} in the aforementioned 5-year series of patients

in whom a clinical diagnosis only was made. In 45 of these 53 patients, the 24-hour uptake of I^{131} was low, varying from zero to 11 per cent, whereas the uptake was normal in the remaining 8 patients, varying from 12 to 43 per cent. The majority of the latter patients had mild or subsiding symptoms.

Of the entire 108 patients, 19 either were frankly hyperthyroid or were suspected of being hyperthyroid. Four of the patients were thought to have had Graves' disease in addition to granulomatous thyroiditis. However, in the other 15 patients suspected of having hyperthyroidism, it was considered that the symptoms of toxicity were due to the release of excessive amounts of thyroidal hormone into the circulation as a result of destruction of thyroidal tissue by the thyroiditis.

The following 3 cases are reported to illustrate the findings in patients who had mild, moderate and severe symptoms, respectively.

REPORT OF ILLUSTRATIVE CASES

Case 1. A 57-year-old woman, first seen in 1950, had noted a lump in her neck for three months. During this period, she had experienced slight pain and tenderness over the region of the thyroid, mild aching up the sides of her neck into her ears, and slight pain in the neck on swallowing. Two or three years before, her home physician had told her he thought she had a small goiter, but she had not been aware of it then.

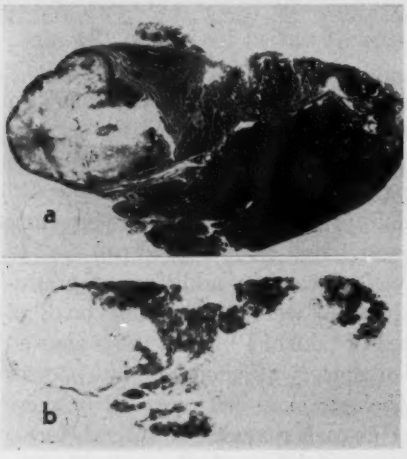

FIG. 3. (Case 1.) Granulomatous thyroiditis with mild symptoms. **a.** Involved lobe, showing focal zone of thyroiditis and single fetal adenoma (hematoxylin and eosin; ×2). **b.** Autoradiogram oriented in same position as **a**, showing no uptake in zone of thyroiditis and good uptake in uninvolved thyroidal tissue.

Each lobe of the thyroid was enlarged to about 3 by 5.5 cm. and was hard and slightly tender. She was euthyroid clinically. A 500-microcurie tracer of I^{131} was given preoperatively for autoradiographic studies. The uptake of I^{131} by the thyroid was 25.6 per cent in twenty-four hours.

The clinical diagnosis was subacute thyroiditis or carcinoma, and subtotal thyroidectomy was performed. Pathologic examination revealed a zone of focal granulomatous thyroiditis measuring approximately 3 by 2 by 1 cm. in the right lobe, as well as a 2-cm. fetal adenoma (Fig. 3*a*). The intervening thyroidal tissue was normal. An autoradiogram revealed no uptake in the zone of thyroiditis but apparently normal uptake in the uninvolved parenchyma (Fig. 3*b*). The left lobe contained a similar smaller focus of thyroiditis.

Case 2. In 1954 a 48-year-old man had noted a tender lump in the right side of his neck for three weeks before admission. He complained of malaise, moderate pain in

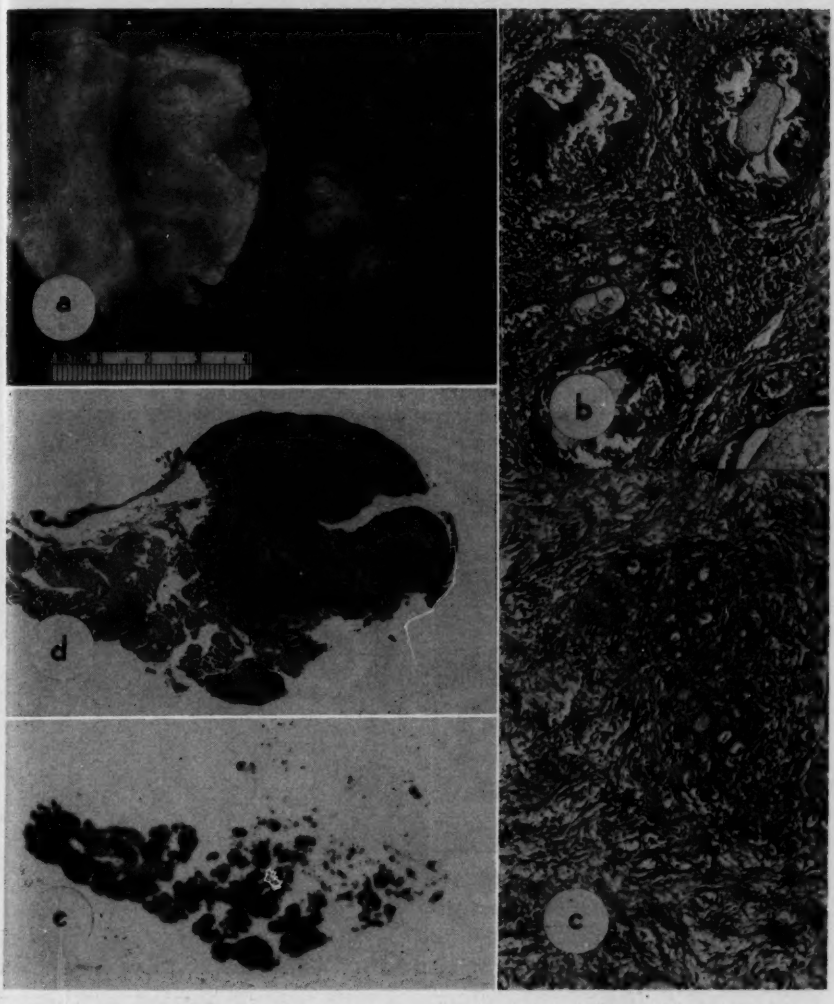

FIG. 4. (Case 2.) Moderately severe granulomatous thyroiditis. **a.** Resected thyroid, showing pronounced involvement of right lobe, with slight extension to the left lobe. **b.** Zone of active granulomatous thyroiditis (hematoxylin and eosin; ×85). **c.** Inactive fibrosed region (hematoxylin and eosin; ×80). **d.** Left lobe, showing granulomatous thyroiditis in upper right portion of picture (hematoxylin and eosin; ×2). **e.** Autoradiogram oriented in same position as **d**, showing uptake in the uninvolved portion.

the neck, especially on swallowing, and moderate tenderness on pressure over the thyroid.

Examination revealed a moderately tender, firm, nodular enlargement of the right

lobe of the thyroid. The left lobe was thought to be abnormal to palpation but involved to a lesser degree. The patient was euthyroid clinically. The basal metabolic rate was −1 per cent and the erythrocytic sedimentation rate was 70 mm. A 300-microcurie tracer of I^{131} was given for gammagraphic studies. The 24-hour thyroidal uptake was 9.1 per cent, but the gammagram showed that almost all the I^{131} in the gland was in the left lobe. The right lobe collected almost none.

Although a diagnosis of subacute thyroiditis was considered, carcinoma was thought to be more likely, and subtotal thyroidectomy was performed. Pathologic examination revealed bilateral granulomatous thyroiditis involving almost the entire right lobe, with a focal zone in the left lobe (Fig. 4*a*). Various stages of activity of the disease were noted in the right lobe, including large areas with central healing and fibrosis and other areas of activity (Fig. 4*b* and *c*). Autoradiograms revealed practically no uptake on the right but apparently normal uptake in the uninvolved portion of the left lobe (Fig. 4*d* and *e*).

Case 3. In 1937 a 39-year-old woman noted a small tender mass in the neck of two

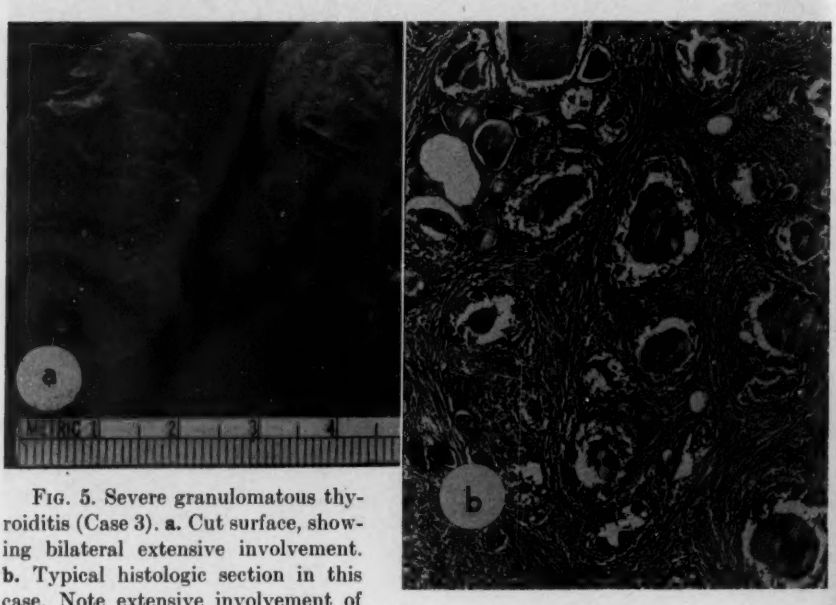

FIG. 5. Severe granulomatous thyroiditis (Case 3). **a.** Cut surface, showing bilateral extensive involvement. **b.** Typical histologic section in this case. Note extensive involvement of thyroidal follicles, each of which contains a zone of inflammatory cells, mainly macrophages, surrounding the colloid (hematoxylin and eosin; ×55).

months' duration. She also complained of severe malaise, fever, pronounced pain on swallowing and pain up the sides of her neck into her ears.

She appeared to be acutely ill and clinically hyperthyroid. The basal metabolic rate was +21 per cent and the erythrocytic sedimentation rate was 118 mm. The thyroid gland was bilaterally enlarged and smooth; each lobe measured about 5 by 4 by 4 cm. and was extremely hard and exquisitely tender.

A diagnosis of acute diffuse thyroiditis was made, and subtotal thyroidectomy was

performed. Gross examination revealed the picture of extensive bilateral granulomatous thyroiditis, with little colloid-containing thyroid visible on cut section of both lobes (Fig. 5*a*). Microscopic study showed a corresponding diffuse involvement with active breakdown of follicles throughout (Fig. 5*b*).

GROSS SURGICAL AND PATHOLOGIC FINDINGS

The gross pathologic findings in granulomatous thyroiditis vary according to the stage of the disease and the extent of thyroidal involvement by the inflammatory process. Inspection at operation usually reveals some degree of inflammatory reaction involving the capsule, and loose or firm adhesions may be present. In this series of 103 surgically explored glands (excluding 5 cases in which needle biopsy only was performed), the thyroid was described as adherent to overlying muscle in 18 cases, and also as "adherent" or "firmly adherent" to other structures such as the trachea or esophagus, in an additional 5 instances. However, the gland practically always can be separated readily from these structures, and adherent muscle is an infrequent finding in the surgically resected tissue. Nothing in the surgical description in any of these cases suggested the invasive fibrosing inflammatory process that is distinctive of Riedel's struma and that renders many of the latter cases "inoperable." The surgical specimens in granulomatous thyroiditis show a corresponding lack of adherent and involved adjacent structures.

Thyroidal enlargement, as described by the surgeon or determined by measurement of resected specimens in this series, was moderate in degree. Most of the involved lobes were approximately one and a half to three times the normal size; exceptionally, a focally or diffusely involved but normal-sized gland was described by the surgeon. The enlargement in 2 instances was considerably greater than average, the glands being estimated as six to eight times the normal size. The thyroid gland was involved bilaterally in 53 of the 108 cases and was involved unilaterally in 54; the disease was limited to the isthmus in 1 instance. When unilateral, the disease tended to affect the right side (38 cases) rather than the left (16 cases). In 4 cases in which the involvement originally was unilateral, the second lobe became affected after a symptom-free period of one to three months; in 1 additional case, this interval was seventeen years. Unilateral involvement was present in 26 of the aforementioned 37 "asymptomatic" patients, and in 35 of the 64 patients with mild or moderate symptoms. All 7 patients with severe disease had bilateral involvement.

Zones of granulomatous thyroiditis usually can be readily recognized grossly, standing out in sharp contrast to the normal thyroidal tissue. The involved regions are tough, firm or resilient, and are white or yellow-white. When focal, the involved region may be demarcated by a fairly sharp

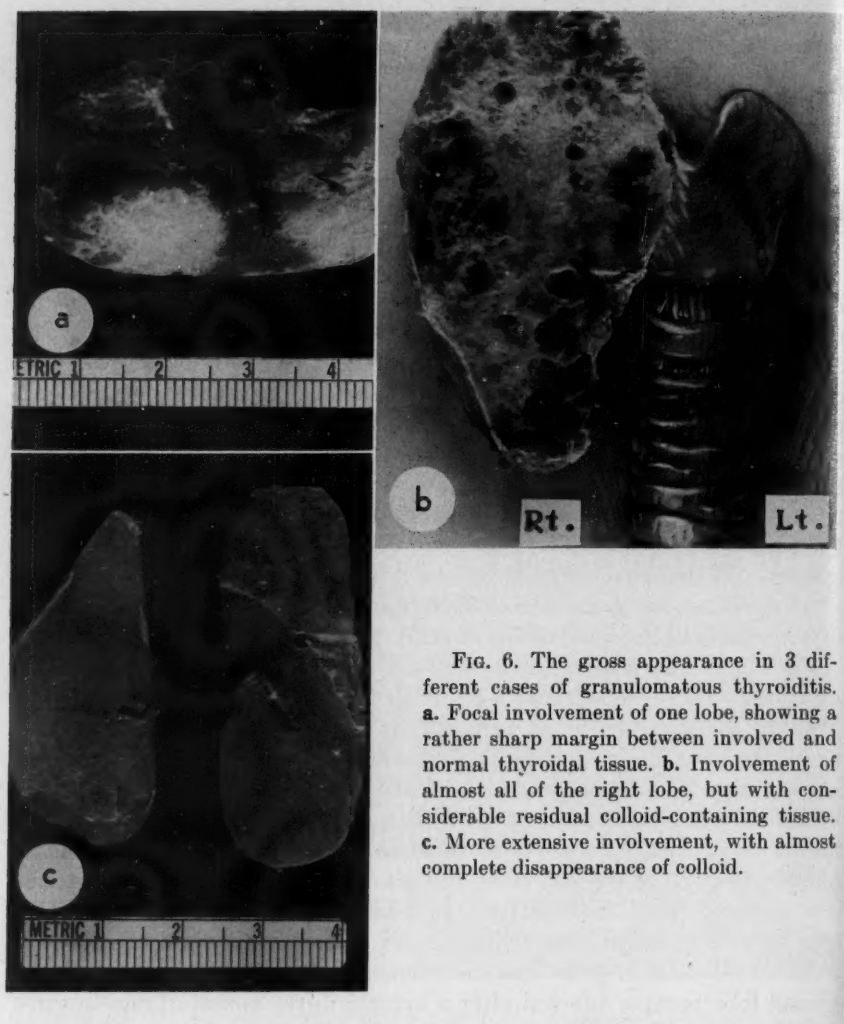

FIG. 6. The gross appearance in 3 different cases of granulomatous thyroiditis. **a.** Focal involvement of one lobe, showing a rather sharp margin between involved and normal thyroidal tissue. **b.** Involvement of almost all of the right lobe, but with considerable residual colloid-containing tissue. **c.** More extensive involvement, with almost complete disappearance of colloid.

margin (Fig. 6*a*) or may blend more imperceptibly into uninvolved colloid-containing zones. More diffuse involvement may appear to extend throughout the entire lobe, leaving scattered foci of persisting colloid-containing tissue (Fig. 6*b*); in other cases, the involvement of the gland may be apparently complete, with little or no colloid-containing tissue (Fig. 6*c*).

The disease apparently develops in normal thyroidal parenchyma, although fetal or colloid adenomas may be present. The associated pathologic

findings in these 108 cases were as follows: single fetal adenoma (2 to 5 cm. in diameter), 9 cases; single colloid adenoma (1 to 2 cm. in diameter), 5 cases; adenomatous goiter (nontoxic), 2 cases; papillary and follicular carcinoma (2 cm. in diameter), 1 case; Graves' disease, 2 cases.

Seven of these 14 adenomas were palpable and were known to have been present for many years; the remainder were small and undetected on physical examination, the palpable lesion being granulomatous thyroiditis in each case. In 2 cases, a localized zone of granulomatous thyroiditis was present in a thyroid removed for Graves' disease. In 2 additional cases, the pathologic findings were those of granulomatous thyroiditis, whereas the clinical picture was suggestive of Graves' disease. Studies utilizing I^{131} were not made in these patients, and it was considered that the presence of Graves' disease in addition to granulomatous thyroiditis could not be established with certainty.

MICROSCOPIC FINDINGS

As already noted, the histologic findings in granulomatous thyroiditis vary according to the stage of the disease. The process may be uniform and apparently at the same stage throughout much of the gland or, more frequently, zones of activity alternate with zones of healing and fibrosis. The early active granulomatous process produces disruption of normal follicular epithelium with residual colloid, apparently undergoing progressive breakdown and phagocytosis, surrounded by a zone of histiocytes and foreign-body giant cells (Fig. 7*a*). In the early active period, the inflammatory cells surrounding the colloid in some follicles may be partially or wholly composed of polymorphonuclear leukocytes, so that micro-abscesses are formed (Fig. 7*b*). These abscesses, although noted in histologic sections in 45 of the 108 cases in this study, are never a prominent feature of the over-all histologic response, so that the old term "acute nonsuppurative thyroiditis" appears to be justified. A somewhat later stage in the process is marked by a fairly pronounced tuberculoid response, with almost complete disappearance of colloid and somewhat more extensive inflammatory fibrosis (Fig. 7*c*). As the inflammatory process subsides still more, there remains a fibrous stroma containing a few inflammatory cells and a variable number of small follicles, usually empty of colloid (Fig. 7*d*).

The healed phase of the disease is illustrated by the following 2 cases.

Case 4. A 61-year-old woman had a typical attack of granulomatous thyroiditis with bilateral involvement, pain, tenderness, referred pain, fever and an erythrocytic sedimentation rate of 97 mm. Treatment with cortisone and x-rays resulted in relief of symptoms, and the gland after two months was nontender and nonpalpable. At necropsy, four years later, the thyroid was found to be small, each lobe measuring 4 by 2 by 0.8 cm. (Fig. 8*a*). There was slight thickening of the capsule, with some old adhesions on

the surface. The cut surface showed increased fibrosis and a rather small amount of residual colloid-containing thyroidal tissue. Histologic examination revealed diffuse fibrosis, both interfollicular and interlobular, with no residual evidence of active inflammation (Fig. 8*b*).

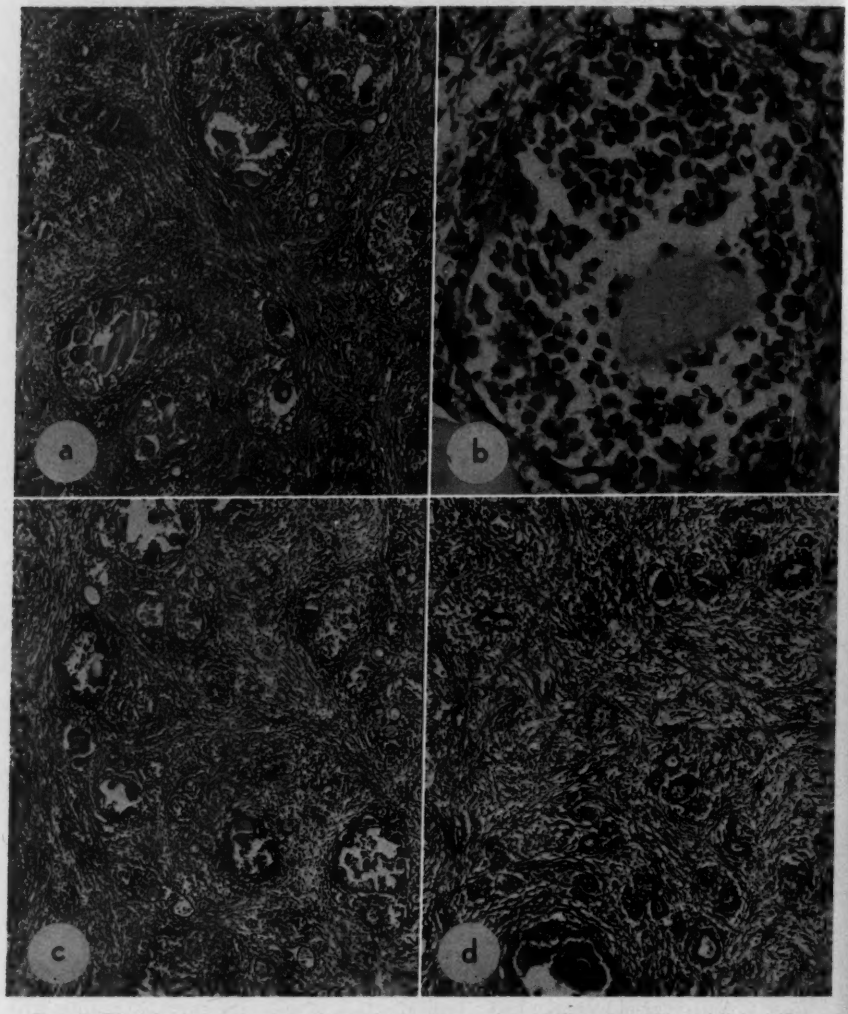

FIG. 7. Histologic appearance in various stages of granulomatous thyroiditis (all sections stained with hematoxylin and eosin). **a.** Active breakdown of colloid, typical giant-cell reaction and associated moderate inflammatory fibrosis (×65). **b.** Polymorphonuclear leukocytes surrounding residual colloid (×360). **c.** Somewhat later stage of the inflammatory process. Most of the colloid has disappeared and there is more abundant fibrosis (×60). **d.** Late stage, showing occasional aggregates of giant cells and extensive fibrosis (×95).

Case 5. A 38-year-old woman at the time of her first admission had a typical acute attack of granulomatous thyroiditis, more severe on the right. The condition subsided completely with medical treatment. Six years later, at a second admission, a nodule was felt in the right lobe. The preoperative diagnosis was hemorrhage into an adenoma. Surgical resection revealed a small adenoma of the right lobe, with residual fibrosis of the lower pole, apparently representing the region of previous thyroiditis (Fig. 8*c*). The left lobe appeared normal.

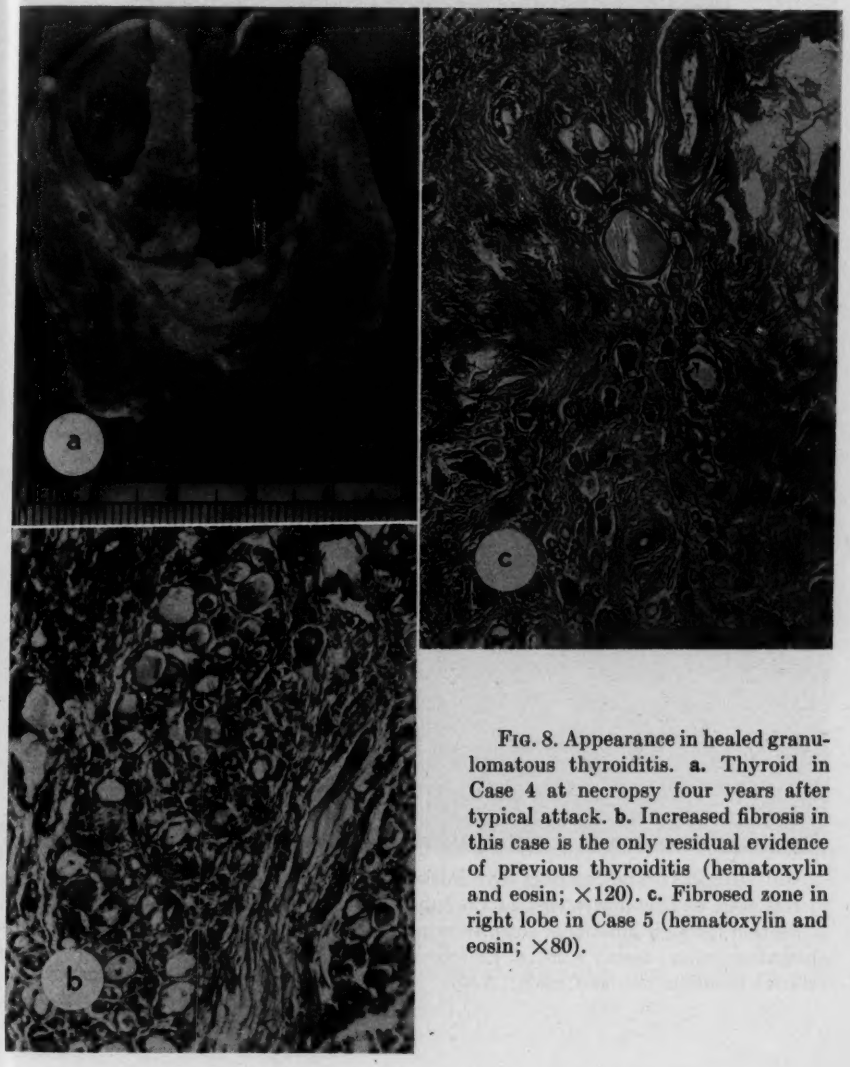

FIG. 8. Appearance in healed granulomatous thyroiditis. **a.** Thyroid in Case 4 at necropsy four years after typical attack. **b.** Increased fibrosis in this case is the only residual evidence of previous thyroiditis (hematoxylin and eosin; ×120). **c.** Fibrosed zone in right lobe in Case 5 (hematoxylin and eosin; ×80).

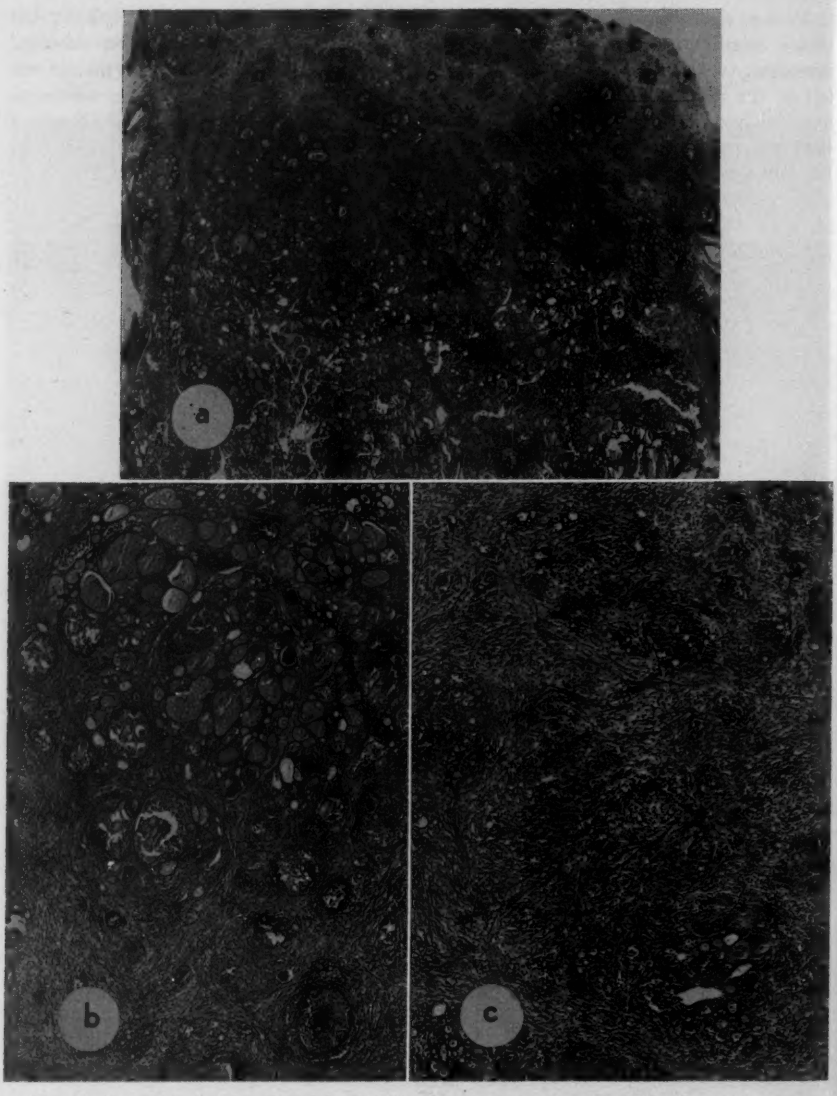

FIG. 9. Focal granulomatous thyroiditis. The 24-hour uptake of I^{131} was 7 per cent in this case. **a.** Low-power view at the junctional zone (hematoxylin and eosin; ×6½). **b.** Section through junctional zone, showing progressive breakdown of thyroidal tissue (hematoxylin and eosin; ×28). **c.** Fibrotic zone in stage of healing. Note small residual follicles (hematoxylin and eosin; ×50).

A large number of lesions appear to be focal in nature, the active destructive inflammatory process spreading peripherally while the center becomes progressively more fibrotic. This process is illustrated in Figure 9, which demonstrates the findings in a case of granulomatous thyroiditis limited to a portion of one lobe. In this case, although the process was extremely active at one particular zone, the total amount of colloid-containing tissue being destroyed at one time was relatively small. At the opposite extreme, there are certain cases in which the disease is extremely extensive and diffuse with active inflammation and destruction of a large part of the thyroidal parenchyma, the process being relatively active in all parts of the gland simultaneously (Fig. 5*b*). An increased basal metabolic rate or symptoms of hyperthyroidism can be reasonably accounted for in such instances by the sudden breakdown and release of a large amount of preformed colloid.

Examination of the uninvolved parenchyma in granulomatous thyroiditis reveals normal thyroidal tissue with rather flattened inactive-appearing epithelium. Nodularity of the hypertrophy-involution type was distinctly unusual in this series of cases. In unilateral or focal disease, the grossly normal parenchyma of the same or opposite lobe may show a scattering of widely separated single follicles or sometimes groups of two or three in which the typical giant-cell reaction is seen. If such singly involved follicles represent "early" involvement, then the giant-cell response must occur at an extremely early stage in the pathologic process.

DIFFERENTIAL DIAGNOSIS

Granulomatous thyroiditis, especially in its localized form, may bear some gross resemblance to invasive papillary and follicular carcinoma, whereas the diffuse form may be confused with the fibrotic type of struma lymphomatosa. The microscopic appearance, however, is diagnostic of the disease. The distinguishing features of Riedel's struma already have been elaborated. The problem in diagnosis for the clinician or surgeon revolves mainly around cases in which the disease is mild or atypical. Asymptomatic patients who have merely a firm or hard mass in the neck should be managed in the same manner as those who have any type of indeterminate nodule in the thyroid, which usually entails surgical exploration to exclude carcinoma. The diagnosis in acute forms of the disease can be readily established by clinical and laboratory data, including studies employing I^{131}. Needle biopsy is rarely feasible at this stage because of the severity of local pain and tenderness. The borderline case will require biopsy or at least extremely close observation until the possibility of carcinoma is excluded.

COURSE AND PROGNOSIS

Granulomatous thyroiditis appears to be a self-limited disease, although occasionally it follows a prolonged course. Information regarding the course of events after dismissal from the clinic was available on 82 of our 108 patients. In 73 patients there was no recurrence of the thyroiditis or continuation of the symptoms of the disease. In 9 patients there was either a continuation of symptoms for some weeks or months after dismissal or a recurrence of symptoms several months after the original attack of thyroiditis has subsided.

Myxedema developed after the thyroiditis in 12 patients in this series. One of these patients had undergone needle biopsy, 2 had had unilateral subtotal resection of a lobe, and the remainder had been subjected to bilateral subtotal resection. Sixty-one patients were known to have remained euthyroid, but the thyroidal state was unknown in the remaining 35 patients.

SUMMARY AND CONCLUSIONS

A study has been made of 108 cases of granulomatous thyroiditis diagnosed pathologically during the 27-year period from 1930 through 1956.

The incidence of granulomatous thyroiditis in current practice at the Mayo Clinic is approximately one eighth that of Graves' disease. It is probably less common than struma lymphomatosa but is at least fifty times more common than invasive fibrous thyroiditis (Riedel's struma).

One third of the 108 patients in this series had no subjective symptoms of the disease other than a lump in the neck; the remaining two thirds had symptoms of varying severity.

Hyperthyroidism was present in 19 patients. Four of these had Graves' disease, whereas the symptoms of toxicity in the remainder were assumed to be due to destruction of thyroidal tissue and release of excessive amounts of thyroidal hormones into the circulation.

Enlargement of the thyroid was moderate, and the process was unilateral in 50 per cent of the cases. Capsular adhesions were sometimes present but there was no invasion of adjacent structures. The thyroiditis appears to develop in an otherwise normal gland, although rarely it may complicate Graves' disease.

Granulomatous thyroiditis is a self-limiting disease, the patient becoming asymptomatic usually in a period of a few months and the thyroid gland becoming non-palpable.

Addendum

Since the completion of this study Eylan, Zmucky and Sheba (16) have published an article in which they suggest that mumps virus was the cause of a recent outbreak of

subacute thyroiditis in Israel. Strong evidence in favor of this hypothesis includes the isolation of a virus which had the characteristics of mumps virus from 2 cases in which thyroid tissue was excised, and the demonstration of a significant and often rising titer of complement-fixing antibodies against mumps virus in 10 of 11 cases. It was also noted that the incidence of mumps was particularly high in Israel during the period in which the cases of thyroiditis were studied. Although the authors considered mumps virus to be the cause of the disease in their patients, they did not exclude the possibility that other etiologic agents are concerned in other localities.

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THE USE OF I^{133} IN THE TREATMENT OF GRAVES' DISEASE*†

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INTRODUCTION

ONE of the first radioisotopes of iodine used to treat hyperthyroidism was cyclotron-produced I^{130} with a half-life of 12.5 hours (1–3). It was not until 1946 that the pile-produced isotope I^{131}, with a half-life of 8.1 days, was made available. Since then, wide experience has been accumulated with this isotope in the treatment of hyperthyroidism. Recent studies (4) have suggested that other radioisotopes of iodine might be used for this type of therapy with varying advantages in decreasing the total body radiation. This study was undertaken to evaluate the possible use of I^{133} for the treatment of hyperthyroidism.

In this study the amount of radiation to the blood following a fixed dose to the thyroid is used as a criterion to determine the relative advantage of the isotope. The rad is used as the unit of radiation dosage. It represents an absorbed dose of 100 ergs of energy per gram, and for tissue it is approximately equal to the roentgen or rep.

In comparing various isotopes on the basis of rads, an assumption is made that the biologic effectiveness in relieving hyperthyroidism is the same for all the radioiodine isotopes if a fixed number of rads is delivered to the gland. Two objections may be raised to this assumption. First, it is known that the radiation from I^{131} beta particles is only about 40 per cent as effective in treating hyperthyroidism as radiation from external x-rays. This may be due to several variables, such as the distribution of radioiodine in the thyroid, variations in follicular function, periodicity in follicular activity, and the range of the beta particles. Since some of these factors may differ for the various isotopes, it is possible that their biologic effectiveness may also differ. Second, the half-lives of the isotopes considered

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TABLE 1. PHYSICAL CONSTANTS OF IODINE ISOTOPES

Isotope	Half-life	Decay constant (per hr.)	Average gamma energy $\bar{E}_\gamma$ (Mev)	Average beta energy $\bar{E}_\beta$ (Mev)	$\epsilon = \frac{\bar{E}_\gamma}{\bar{E}_\beta}$
I^{124}	4.0 days	0.00722	2.01	0.819	2.45
I^{126}	13.0 days	0.00222	0.395	0.353	1.12
I^{130}	12.5 hours	0.0555	2.25	0.283	7.95
I^{131}	8.1 days	0.00356	0.397	0.195	2.04
I^{133}	21.0 hours	0.0330	0.581	0.484	1.20

range from 12.5 hours to 13 days. To deliver the same number of rads to the thyroid, the irradiation rate for an isotope with a short life must be higher than that for an isotope with a long life. It is likely that various irradiation rates do not have the same biologic effectiveness.

METHODS

In calculating the radiation dose to the thyroid and to the blood, the fractional amount of radioiodine in the blood and thyroid as a function of time, the size of the thyroid, the beta and gamma energies, and the half-lives of the isotopes were considered. In the case of the dose to the thyroid, the contributions from beta particles and gamma rays originating in the thyroid were included in the calculations. In calculating the dose to the blood, the contributions from beta particles in the blood and all the gamma rays originating in the body were included. The equations used are given by Berman and co-workers (4). A list of the isotopes considered, with their physical properties, are shown in Table 1.

Eight patients were treated for Graves' disease with I^{133}. This isotope was first used on patients with a rapid rate of release of radioiodine from the thyroid and a serum PBI^{131} level higher than 1 per cent of the dose per liter. The calculated radiation to the blood of these patients was distinctly lowest when I^{133} was employed. There were 5 patients of this type, designated as Group A in Table 2. Later, as experience with the

TABLE 2. DATA ON 2 GROUPS OF PATIENTS TREATED WITH I^{133}

	Group A	Group B
Number of patients	5	3
Maximum thyroidal uptake	82–92%	40–74%
Serum PBI^{131} at 24 hrs.	1.36–4.82%/liter	0.2–0.8%/liter
Rads to thyroid	6,000–18,000	7,000–13,000
Rads to blood	6–50	3–6
Blood rads from I^{133} relative to I^{131}	0.37–0.85	0.53–1.03
Blood rads from I^{133}–I^{131} mixture relative to I^{131}	0.48–0.95	0.66–1

TABLE 3. RATIO OF BLOOD DOSE TO THYROID DOSE FOR VARIOUS IODINE ISOTOPES, RELATIVE TO I^{131}

Patient	Hyperthyroid patients					
	I^{124} $T_{\frac{1}{2}}$*=4 days $\bar{E}_\gamma/\bar{E}_\beta$=2.45	I^{126} 13d 1.12	I^{130} 12.5 hrs. 7.95	I^{131} 8.1d 2.04	I^{133} 21.0 hrs. 1.20	I^{131} / I^{133} mixture
B.R.	.96	1.07	1.04	1	.87	.92
C.L.	1.08	.84	1.45	1	.66	.79
C.R.	.93	.91	.74	1	.59	.73
M.A.	.80	1.19	.51	1	.38	.48

* Half-life.

isotope was gained, 3 patients with a more usual pattern of iodine metabolism were added to the study and are designated as Group B.

A kinetic study of the metabolism of radioiodine was made on each patient before treatment with I^{133}. This consisted of an intravenous injection of about 0.1 millicurie of I^{131}, following which multiple readings were made over the neck with a collimated scintillation counter, and blood samples were taken at one, two, three, four, five and seven hours, twice on the second day and once daily thereafter for a total of ten days. Fractional urine samples were collected for the first forty-eight hours.

From the kinetic data, the dosage in rads per millicurie to the thyroid and the blood was calculated for I^{133}, I^{131}, and other iodine isotopes. The number of millicuries of a given I^{131}-I^{133} mixture necessary to deliver a desired dose to the thyroid was then calculated. The number of millicuries actually given to the patients, however, was deliberately less than the calculated number, in anticipation of a possibly higher biologic effectiveness from I^{133}. This was a precautionary measure to avoid overtreatment.

Multiple measurements of I^{133} in the thyroid and the blood were made during therapy. The radiation dose to the thyroid was calculated from the therapy data. In most cases calculations agreed within 10 to 20 per cent of those predicted from the preceding kinetic study.

I^{133} can be obtained as a fission product. Eleven hours after it is removed from the atomic pile (5), the radioiodine contains about 39 per cent I^{135} (6.7 hour half-life), about 57 per cent I^{133}, and about 4 per cent I^{131}. When used one to two days later, the I^{135} content has become negligible and the I^{131} content has reached about 10 to 25 per cent.

Measurements of I^{133} *in vivo* were made using both a gamma-ray spectrometer and conventional scintillation equipment. Urine and blood samples were measured in the same well-type scintillation counter that is used for I^{131}. Appropriate I^{133} standards were used in each case.

RESULTS

Based on the assumption that the kinetics of iodine are the same for all iodine isotopes, calculations of the dose to the blood for a desired number of rads to the thyroid were made for several iodine isotopes. The results in 4 patients are shown in Table 3 and are expressed relative to I^{131}. Calcula-

tions indicated that I^{133} (half-life, 21 hours) would deliver the lowest blood dose. In 1 patient the factor for the reduction in radiation to blood was as high as 3.

Plots of typical I^{131} tracer data for patients in Groups A and B are shown in Figure 1. Group A patients had very high and rapid thyroidal uptakes

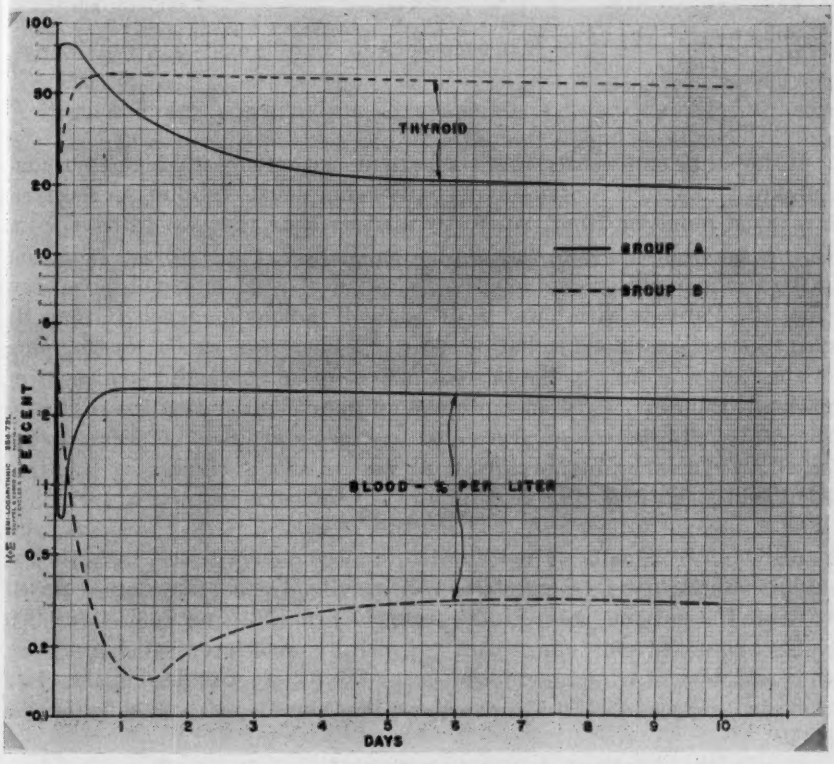

FIG. 1. Kinetics of radioiodine in hyperthyroidism, following a tracer dose of I^{131}. Typical curves for the patients with very high thyroidal uptakes and serum PBI^{131} levels (Group A) are compared with typical curves for patients with a more usual pattern of iodine metabolism in hyperthyroidism (Group B).

and very high levels of serum protein-bound radioiodine. Group B patients had average uptakes and PBI^{131} levels characteristic of hyperthyroidism. The range of values for each group is shown in Table 2. The rads listed were calculated from the I^{133} therapy data. Because the I^{133} had about 10 to 20 per cent of I^{131} in it, the relative advantage of the mixture was not as great as that of pure I^{133}.

TABLE 4. RESULTS OF I^{133} THERAPY IN 8 PATIENTS WITH GRAVES' DISEASE

	Number	Rads received
Patients treated	8	6,000–18,000
Doses given	11	6,000–13,000
Received two doses	3	12,000–18,000
Remission	3	7,000–13,000
Still toxic	1	9,000
Hypothyroid	1	7,000
Too soon to evaluate	3	7,000–18,000

It should be emphasized that the patients with unusually high thyroidal uptakes and serum protein-bound radioiodine levels (Group A) received less blood radiation from the I^{133}-I^{131} mixture than they would have from I^{131}. As may be seen from the last line in Table 2, the I^{133} blood radiation in 1 patient in this group was less than half of what it would have been from I^{131}. Patients with lower protein-bound radioiodine levels (Group B) also received less blood radiation with I^{133}. There was similar overlapping of the ranges of rads-to-the-thyroid necessary for successful treatment of patients of both types. Hence, for the purpose of presenting the results of the study, all 8 patients are grouped together in Table 4 The results are based on clinical and laboratory findings.

DISCUSSION

Although 8 patients do not constitute an adequate series, nevertheless certain conclusions are suggested by the results. The average dose required to treat the patients successfully, using I^{133}, ranged from 7,000 to 18,000 rads—about the same range that is generally considered satisfactory for the treatment of hyperthyroidism, using I^{131} (6). It would seem therefore that, on a rad basis, I^{133} may have about the same therapeutic effectiveness as I^{131}.

It must be emphasized, however, that the results of this comparison are not conclusive. It is well known that the number of rads necessary to treat hyperthyroid patients with I^{131} varies over a wide range. Furthermore, it is still difficult to predict for any one patient the number of rads to the thyroid that will be required for successful treatment. In order to make comparison between I^{133} and I^{131} more meaningful, it is necessary either to have a very large series of patients treated with both isotopes, or to obtain some means whereby hyperthyroid patients can be differentiated on the basis of radiation response, so that successful treatment of a patient can be predicted within closer limits.

CONCLUSIONS

From theoretical consideration alone, general conclusions may be drawn about the advantages of various iodine isotopes in minimizing total body irradiation: 1) an isotope with a lower average gamma-to-beta energy ratio delivers less radiation to the blood for a desired dose to the thyroid, and 2) an isotope with a physical decay that properly matches the biologic metabolism of radioiodine is most desirable. Since the kinetics of radioiodine may be different for each patient, and since conditions 1) and 2) may not be compatible, the same isotope may not be best in all cases.

From the results in the 8 patients treated, it seems that I^{133} has about the same biologic effectiveness as I^{131} for the same number of rads delivered to the thyroid.

In hyperthyroid patients with very high thyroidal radioiodine uptakes and high serum concentrations of protein-bound radioiodine, the radiation to the blood may reach levels as high as 50 or 100 rads when I^{131} is used. Significant reductions in the dose to the blood of some of these patients may be obtained with I^{133}.

SUMMARY

1. Various radioiodine isotopes have been considered in order to determine their possible usefulness in reducing radiation to the blood in patients treated for hyperthyroidism.

2. Of the isotopes considered, I^{133}, with a 21-hour half-life, seemed most promising.

3. Eight patients were treated with I^{133} and required doses of about 7,000 to 18,000 rads. Apparently the biologic effectiveness of I^{133} in the treatment of hyperthyroidism is not radically different from that of I^{131} on a rad basis.

4. Treatment of hyperthyroidism with I^{133} is feasible and will reduce radiation to the blood by a significant amount in some patients.

Acknowledgment

We wish to thank the Brookhaven National Laboratories, and especially Mr. W. D. Tucker for making this isotope available to us on very short notices and irregular schedules. We also wish to thank Mr. Eladio Nunez, Mrs. Charlotte Marfey and Mr. Nathaniel Griffin for their expert technical assistance.

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THYROIDAL UPTAKE AND PLASMA CLEARANCE OF I^{131} AND I^{127} IN CIRRHOSIS OF THE LIVER

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IN A series of 49 patients with cirrhosis of the liver reported by Mueller and associates the 24-hour thyroidal uptake of I^{131} exceeded 50 per cent in nearly half the cases (1). High values were observed most frequently when liver damage was severe. Neither goiter nor other evidence of thyroid hyperfunction could be demonstrated in any patient. Scazziga and coworkers were unable to detect any elevation of thyroidal I^{131} uptake in 10 patients with cirrhosis and 19 with hepatitis (2).

A search of our files revealed that 2 of 5 patients with cirrhosis, referred for routine radioiodine studies, had thyroidal uptakes exceeding 50 per cent. In order to obtain more information about the mechanism involved, and to learn whether the results in a larger series would confirm this high incidence, special studies were made on 36 additional patients with a well established diagnosis of hepatic cirrhosis.

CLINICAL DATA AND METHODS

Patients

The 36 patients were males ranging in age from 32 to 67 years. A positive history of severe alcoholism was obtained in 33 of the 36. In the remaining 3, biopsy revealed post-necrotic cirrhosis in 1, and portal cirrhosis in the other 2. Presenting symptoms almost invariably included adbominal distention caused by ascites. Most of a series of tests for liver impairment, such as cephalin flocculation, zinc sulfate turbidity, bromsulfalein retention, and serum bilirubin, yielded abnormal results. The liver was invariably palpable and was firm in consistency. No patients with clinical evidence of thyroid abnormality nor any who had received iodine-containing compounds in the preceding three months, were included for study.

The control series comprised selected male patients with minor complaints, along with male hospital personnel of comparable age.

Methods

A dose of 50 microcuries of I^{131} was administered orally approximately two hours after breakfast. Uptake over the thyroid at one hour was measured at a distance of 50 cm. with a collimated NaI scintillation counter fitted with a 1/16-inch lead filter (3). A thigh reading was subtracted. Readings were made again at two, three, six, and twenty-four hours. A blood sample was drawn at two hours and again at twenty-four hours. The bladder was emptied at one hour, after which separate collections of urine were made between one and two hours, and two and three hours. Three glasses of water were given between the first and third hour to promote the flow of urine.

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The I^{127} content of the two separate urine samples was determined by a modification of the method of O'Neal and Simms (4). An average of the two was taken as hourly output. The amount of urine assayed was usually 0.5 ml., but in order to bring the colorimeter readings near the middle of the standard curve, *i.e.*, 0.02 to 0.04 microgram of I^{127}, re-estimation was sometimes necessary with quantities between 0.25 and 2.0 ml. Protein-bound I^{127} of serum (PBI) was determined by a modification of the alkaline ashing method of Barker (5).

Urinary I^{131} content was measured in a NaI scintillation well counter.

Radioactivity of serum was determined with a thin-window Geiger tube. A 1-ml. sample in a 1-inch steel counting cup with a disc of lens tissue on the bottom was dried in the oven at 80° C. after the addition of one to two drops of 10 per cent Tween 20, 8 drops of concentrated ammonium hydroxide, and 4 drops of 1/50 *M* palladium chloride in 1/50 *M* hydrochloric acid. An aliquot of nonradioactive serum containing 0.002 per cent of the patient's dose treated in the same way served as a standard.

A thrice washed zinc sulfate-sodium hydroxide precipitate from 1 ml. of serum was employed in the determination of the PBI^{131} conversion ratio. The precipitate was flushed with 28 per cent ammonia water into a counting cup, stabilizing reagents added as with serum, and drying accomplished under a heat lamp. The dry precipitate finally was flattened onto the bottom by crushing into fine granules under acetone. Counts of the sample and of standard I^{131} added to a similar precipitate were obtained under a Geiger tube.

Calculations

$$\text{I. Conversion ratio} = \frac{\text{count } PBI^{131} \text{ precipitate}}{\text{count whole serum}} \times 100$$

$$\text{II. Thyroidal } I^{131} \text{ clearance in ml. plasma/min.} = \frac{(\%\text{ dose into thyroid in 3 hrs.}) - (\%\text{ in 1 hr.})}{(120\text{ min.}) \times (\%\text{ dose per ml. serum at 2 hrs.})}$$

Assumption was made that essentially all radioactivity at two hours consisted of inorganic iodide. Justification for this assumption was obtained from accumulated data relating to the percentage of radioactivity in the protein-bound fraction at this early time. The protein-bound fraction averaged only 2.5 per cent of the total for 36 euthyroid subjects and 6 per cent for a similar number of hyperthyroid patients.

$$\text{III. Thyroidal } I^{127} \text{ uptake } (\mu\text{g. per 24 hrs.}) = \frac{(\%\text{ dose } I^{131} \text{ into thyroid between 1 and 3 hrs.}) \times (12) \times (\mu\text{g. } I^{127}\text{/ml. urine})}{\%\text{ dose/ml. of urine}}$$

It was assumed that the mean specific activity of the two urine samples was the same as that of blood plasma during this time, and that the radioactivity of the blood sample taken at the mid-point represented an approximate mean for the interval. Assumption also was made that urinary iodine for practical purposes consists entirely of iodide.

$$\text{IV. } \mu\text{g. } I^{127} \text{ per ml. serum} = \frac{(\%\text{ dose } I^{131} \text{ per ml. serum}) \times (\mu\text{g. } I^{127}\text{/ml. urine})}{\%\text{ dose/ml. urine}}$$

RESULTS

The values identified by letter in Figure 1 represent individuals in whom values exceeded the upper limits of normal in at least one of the four

test-procedures indicated in the four columns. These 8 subjects comprised 22 per cent of the total series of cirrhotics. All were cases of severe alcoholism.

In 5 of these patients the 24-hour thyroidal uptake exceeded the upper limit of 45 per cent reached by the control group. There were 7 patients

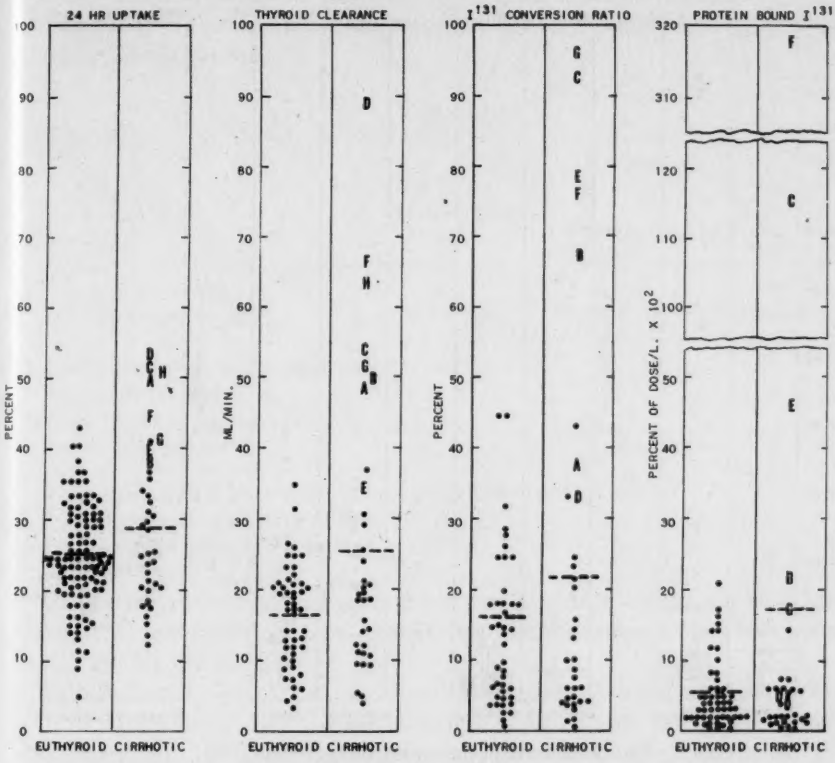

FIG. 1. Results of tests with radioiodine (I^{131}). The broken horizontal lines represent the mean for the group.

with clearances exceeding 40 ml./min.—approximately 19 per cent of the total series. The mean was 25.5 ml./min. ±3.3, as contrasted with 16.6 ml./min. ±1.0 for the controls (P = 0.01). The conversion ratios in 5 cirrhotic subjects were well above the normal range. A conversion ratio was not determined in Patient H. Patient E, in whom results were on the borderline by other tests, had a very high ratio. Column 4 of Figure 1 depicts absolute values for the protein-bound I^{131} activity of serum at twenty-four hours.

Although the disparity from normal is not so striking as it is for the conversion ratio, 4 determinations were distinctly high.

Portions of serum obtained at twenty-four hours from Patients A through E were extracted by a butanol-alkali procedure in order to verify the high ratio of hormonal to total I^{131} activity shown by the PBI^{131} con-

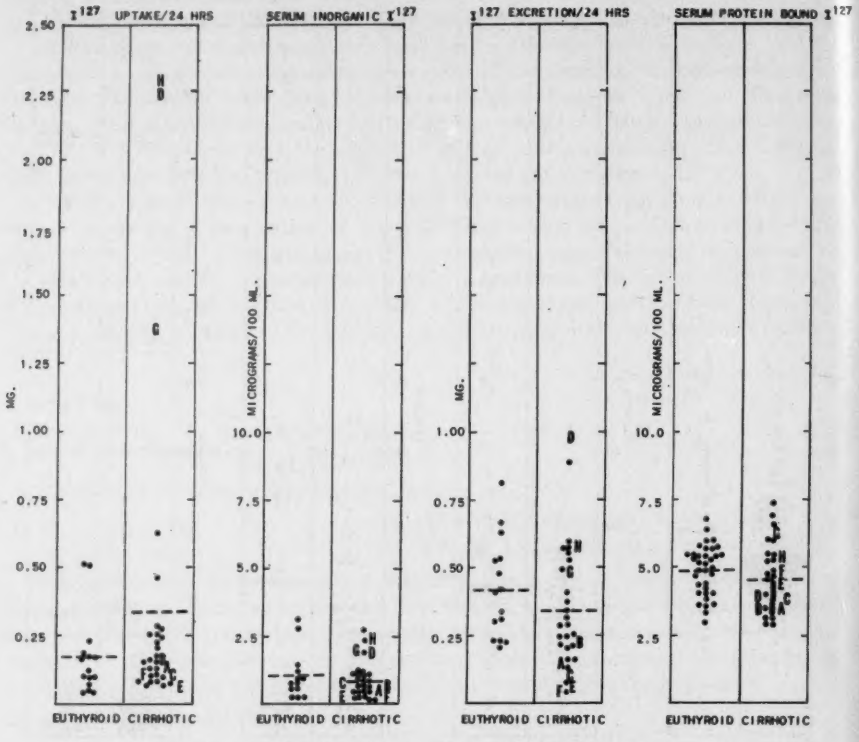

FIG. 2. Results of tests with stable iodine (I^{127}).

version ratio. Respective values were 32, 61, 66, 31, and 60 per cent. These exceed the normal upper limit of 26 per cent obtained from a separate series of 45 selected euthyroid subjects.

The complete series of I^{131} tests was repeated in Patients C, F and H after one to two months in the hospital. All results were then normal.

Observations plotted in the first column of Figure 2 represent the thyroidal uptake of I^{127} in cirrhotic and control subjects. Of the 8 patients previously singled out by I^{131} studies, 3 had a high uptake of I^{127}. In these 3 subjects the serum inorganic I^{127} concentration (column 2) and urinary

excretion of I^{127} (column 3) also were greater than average. In the remaining 5 the uptake of I^{127} was below average. This is not unexpected, because even though clearance was rapid the plasma level of I^{127} was relatively low. Excretion also was lower than the mean of either the cirrhotic or the normal group. A total of 9 cirrhotic subjects excreted less than the lowest control subject. Because samples were collected postprandially, most values for I^{127} in urine and serum undoubtedly were somewhat higher than the true 24-hour mean.

TABLE 1

	Intake of sodium		
	Unrestricted salt	1,200 mg. Na	400 mg. Na
I^{127} excretion, mg./24 hrs., (mean and range)	0.42 (0.15–0.89)	0.32 (0.05–0.58)	0.29 (0.06–0.96)
No. of patients having normal results with I^{131} tests*	9	10	9
No. of patients having abnormal results with I^{131} tests*	1	3	4

* Days in hospital prior to I^{121} study (mean and range):
Normal group, 31 days (2–120).
Abnormal group, 15 days (4–28).

Of particular interest in Figure 2 is column 4. All cirrhotic subjects had serum PBI levels below 7.5 μg. per 100 ml. The mean was 4.6 μg. compared to 4.9 μg. for the controls.

Approximately one third of the subjects with cirrhosis were receiving a full diet seasoned with iodized salt, but in two thirds the intake of salt was restricted. The interrelationships of salt intake, iodine excretion, I^{131} test data, and length of hospitalization before performance of thyroid studies are presented in Table 1. Although definitive statistical conclusions are not justified, certain trends are observable. For one, the excretion of iodine tended to vary with the degree of salt restriction. Two subjects receiving 400 mg. of sodium, who for unknown reasons excreted large amounts of iodine, distorted the mean for this group. The remainder of the excretions varied between 0.06 and 0.28 mg. Other trends were the higher proportion of abnormal I^{131} test results among patients whose intake of salt was restricted, and the shorter mean period of antecedent hospitalization in the group with abnormal I^{131} test results.

DISCUSSION

That the amount of I^{131} taken up by the thyroid gland per unit time is proportional not only to the functional activity of the gland but also to the plasma concentration of I^{131} was first shown by Myant, Pochin and Goldie (6). Because the level of radioactive iodide in plasma is affected by the rate of renal removal and by extra plasma dilution, a cumulative 24-hour uptake is not related solely to thyroid function. An estimate of clearance, on the other hand, provides a direct measure of the iodide-accumulating function of the gland, because the method is self-corrected for variations in plasma concentration. For this reason the 7 subjects with high clearance rates represent examples of true hyperavidity of the thyroid for iodide.

Iodide uptake in itself is not a measure of total thyroid function. In none of the patients with high rates of clearance was there clinical evidence of hyperthyroidism. Moreover, the normal serum PBI values speak strongly against hypersecretion of hormone. That hypersecretion might be masked by accelerated peripheral utilization seems unlikely. Neither is it plausible that a high rate of secretion was not mirrored in the PBI determinations because of a low concentration of plasma protein. Total plasma protein levels determined in the entire series of cirrhotic patients prior to the thyroid studies ranged from 5.5 to 8.5 Gm. per 100 ml., and averaged 6.8 Gm. The range in the 8 patients with elevated I^{131} clearance rates was 6.2 to 7.7 Gm. per 100 ml., with a mean of 6.9 Gm.

Accelerated thyroidal uptake dissociated from augmented hormone secretion is a classic finding in patients suffering dietary iodine deficiency (7, 8). The possibility of such a disorder complicating hepatic cirrhosis should be seriously considered. A similar suggestion has been made by Berson (9). Because a dietary history is of uncertain reliability in alcoholic patients, any attempt to correlate I^{131} uptake with individual dietary habits is futile. Nevertheless the presence of cirrhosis itself favors a severe pre-existing nutritional deficiency. Even if the patients had chosen to use iodized salt, their daily quota of nonalcoholic food might not have been adequate to require the use of salt.

To be emphasized is the finding of Stanbury and co-workers that I^{131} uptake, elevated because of iodine deficiency, remains high for weeks after adequate amounts of the element are added to the diet (7). Insult to the thyroid from a deficient iodide supply is not rapidly repaired. While repair is progressing, normal plasma levels of I^{127} will promote a high clearance of I^{127} in addition to I^{131}.

That hyperavidity for iodide in the cirrhotic patient seems to be ameliorated by lengthy hospitalization is suggested by the data in Table 1. Confirming the effect of hospitalization is the reversion of clearance and con-

version ratios to normal when the tests were repeated in Patients C, F and H one to two months later. Even while receiving a low-salt diet, patients may in the course of time obtain enough natural iodine in the food to restore thyroid function to normal. Iodine excretion was not negligible in the patients receiving the restricted diets (Table 1).

If dietary iodine deficiency is the cause of the high thyroidal I^{131} uptake in certain patients with cirrhosis, why is a goiter not present? Mueller and co-workers found no increase in the weight or cell height in thyroid glands obtained at autopsy from 2 cirrhotic patients whose uptake had previously been high (1). Nevertheless, the anatomic observations may have followed a lengthy period of hospital therapy during which iodine balance was regained. I^{131} uptake in 1 of the 2 patients had declined to normal at the time of a second determination made shortly before death. Pertinent to the question of whether goiter might be expected is the observation of Roche and co-workers that I^{131} uptake may be high among inhabitants of an iodine-deficient area even in the absence of goiter (8). Elevation of I^{131} uptake appears to be a more sensitive index of iodine deficiency than are goitrogenic manifestations.

The high conversion ratios in Patients B, C, E anf F can be ascribed at least in part to an absolute elevation of hormonal I^{131} levels, rather than to accelerated iodide removal or expanded iodide space alone. An absolute elevation of the hormonal fraction, nevertheless, does not necessarily prove hypersecretion. Elevation has been observed in known cases of iodine deficiency (8, 10). A high value for serum PBI^{131} also has been reported by Paley, Sobel and Yalow in a case of chronic alcoholism (11). Their suggestion that the cause might have been a depleted organic iodine pool seems attractive. The secretory product of a gland with a small hormonal pool would contain a relatively high concentration of I^{131} irrespective of the secetory rate.

SUMMARY

High values for either thyroidal uptake of I^{131}, clearance, or conversion ratio were demonstrated in 8 of 36 patients with cirrhosis of the liver. Serum PBI^{131} values were normal in all subjects. A pre-existing dietary deficiency of iodine best explains the occurrence of enhanced thyroidal avidity for iodide in certain cases of alcoholic cirrhosis.

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17-α-HYDROXYPROGESTERONE ACETATE; AN EFFECTIVE PROGESTATIONAL SUBSTANCE ON ORAL ADMINISTRATION

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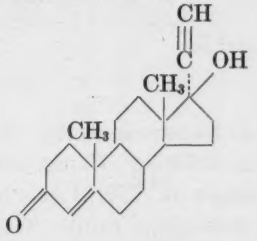
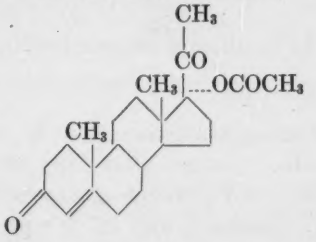
PROGESTERONE is most effective when administered parenterally and it is rather rapidly metabolized and excreted. In order to avoid daily injections of progesterone, it is necessary to have either a parenteral form with a prolonged effect (1, 2) or an oral substance with progestational properties. The progestational substance most widely used for oral administration is 20,21-anhydro-17-β-hydroxyprogesterone (pregneninolone, 17-α-ethinyltestosterone).

It is the purpose of this paper to introduce and describe a new steroid for oral administration, 17-α-hydroxyprogesterone acetate*, and to compare it with the most widely used oral substance with progestational properties, 20,21-anhydro-17-β-hydroxyprogesterone.

20,21-anhydro-17β-hydroxyprogesterone (pregneninolone, 17α-ethinyltestosterone) ($C_{21}H_{28}O_2$)



17-α-hydroxyprogesterone-17-acetate (17-α-hydroxy-4-pregnene-3,20-dione acetate) ($C_{23}H_{32}O_4$)



PATIENTS AND METHOD

Comparative tests were performed on 3 surgically castrated women, who previously had been subjected to other cyclic studies during which it was shown that secretory changes could be induced in the endometria.

The tests were performed with anhydrohydroxyprogesterone, and with 17-α-hydroxyprogesterone acetate. For the purpose of producing proliferation, the dosage of estrogen was always 1 mg. of diethylstilbestrol daily (orally) for the complete 29-day cycle. Either anhydrohydroxyprogesterone or 17-α-hydroxyprogesterone acetate was administered from the fourteenth through the twenty-ninth day. The exact dosages are shown in Table 1.

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* Prodox, Upjohn Co., Kalamazoo, Michigan.

TABLE 1

No. of test cycle	Patient	Daily dosage (oral) of diethylstilbestrol for 30 days preceding test (mg.)	Daily dosages (oral) of 17-α-hydroxyprogesterone acetate from 15th day on:	Daily dosages (oral) of anhydrohydroxyprogesterone from 15th day on:	Day of endometrial biopsy	Days during which bleeding occurred
1	Surgical	1	50mg.×15d		31	32–36
2	castrate	1		50mg.×15d	31	32–36
3	No. 1	1		150mg.×15d	31	31–35
4	Surgical	1	25mg.×15d		31	31–35
5	castrate	1	75mg.×15d		31	31–35
6	No. 2	1		75mg.×15d	31	31–36
7		1		200mg.×15d	31	32–37
8	Surgical	1	50mg.×15d		30	31–36
9	castrate	1	100mg.×15d		30	31–35
10	No. 3	1		100mg.×15d	30	31–35
11		1		250mg.×15d	30	31–36

RESULTS

The results are summarized in Tables 2 and 3.

Endometrium

Histologic examination of endometrial biopsy material showed no secretory changes after daily administration of 50 mg., 75 mg. and 100 mg. of anhydrohydroxprogesterone (total dosages of 750, 1,125 and 1,500 mg.) (Tables 2 and 3). A representative example of results with 100 mg. daily is presented in Figure 1. Rare glands exhibiting secretory changes in endometrium with cystic hyperplasia could be observed after daily administration of 150 mg. of anhydrohydroxyprogesterone (total dosage, 2.25 Gm.) and 25 mg. of 17-α-hydroxyprogesterone acetate (total dosage, 375 mg.). Early secretory activity or variable secretory changes could be observed after daily administration of 200 mg. of anhydrohydroxyprogesterone (total dosage, of 3 Gm.) or 50 mg. of 17-α-hydroxyprogesterone acetate (total dosage, 750 mg.). Definite secretory changes in all glands could be observed after daily administration of 250 mg. of anhydrohydroxyprogesterone (total dosage, 3.75 Gm.) or 75 mg. of 17-α-hydroxyprogesterone acetate (total dosage, 1,125 mg.). As shown in Figure 2, secretory changes characteristic of the secretory phase of the normal menstrual cycle

TABLE 2. 17-α-HYDROXYPROGESTERONE ACETATE

Daily dosage for 15 days (mg.)	Endometrium	Vaginal epithelium	Cervical mucus	Basal body temperature
25	proliferative; moderate cystic hyperplasia	no regression	no inhibition	no rise
50	early secretory activity	moderate regression	partial abolition	rise
50	variable secretory changes	moderate regression	abolition	rise
75	definite secretory changes	marked regression	abolition	rise
100	typical secretory changes	either marked regression or typical luteal changes	abolition	rise

TABLE 3. ANHYDROHYDROXYPROGESTERONE

Daily dosage for 15 days (mg.)	Endometrium	Vaginal epithelium	Cervical mucus	Basal body temperature
50	proliferative; moderate cystic hyperplasia	no regression	(not examined)	(not determined)
75	proliferative	no regression	(not examined)	(not determined)
100	proliferative; slight cystic hyperplasia	no regression	no inhibition	no rise
100	rare glands with secretory changes and cystic hyperplasia	slight regression	no inhibition	no rise
200	early secretory activity	slight regression	partial abolition	no rise
250	definite secretory changes	moderate regression	abolition	no rise

A
B

were observed after daily administration of 100 mg. of 17-α-hydroxyprogesterone acetate (total dosage, 1.5 Gm.).

Vaginal epithelium

Cytologic examination of the vaginal epithelium showed no regression as a result of daily administration of 50 mg., 75 mg. and 100 mg. of anhydrohydroxyprogesterone or 15 mg. of 17-α-hydroxyprogesterone acetate (Tables 2 and 3). Slight or moderate regression was observed after daily administration of 150 mg., 200 mg. and 250 mg. of anhydrohydroxyprogesterone or 50 mg. of 17-α-hydroxyprogesterone acetate. A picture ranging from marked regression to characteristic luteal changes was observed after daily administration of 75 mg. or 100 mg. of 17-α-hydroxyprogesterone acetate.

Cervical mucus

There was no inhibition of the fern-like patterns of the dried cervical mucus as a result of daily administration of 100 mg. and 150 mg. of anhydrohydroxyprogesterone or 25 mg. of 17-α-hydroxyprogesterone acetate (Tables 2 and 3). Partial abolition was observed as the result of daily administration of 200 mg. of anhydrohydroxyprogesterone; it was also observed in 1 of 2 patients during daily dosage with 50 mg. of 17-α-hydroxyprogesterone acetate. Complete abolition of fern-like patterns was noted after administration of 250 mg. of anhydrohydroxyprogesterone, and in 1 out of 2 cases during daily dosage with 50 mg., 75 mg. or 100 mg. of 17-α-hydroxyprogesterone acetate.

Basal body temperature

No rise of the body temperature was observed in the test cycles induced either with anhydrohydroxyprogesterone or with daily administration of 25 mg. of 17-α-hydroxyprogesterone acetate (Tables 2 and 3). The body temperature rose significantly after daily administration of 50 mg., 75 mg. or 100 mg. of 17-α-hydroxyprogesterone acetate.

CONCLUSIONS

1. 17-α-Hydroxyprogesterone acetate is an orally effective progestational substance, since it (a) induces secretory changes in the endometrium, (b) induces luteal changes in the exfoliated vaginal epithelial cells, (c)

FIG. 1. Proliferative endometrium with areas of hyperplasia after daily administration of 100 mg. of anhydrohydroxyprogesterone (total dosage 1.5 Gm.) (test cycle No. 10): (A) Low-power view showing elongation and irregular dilatation of glands. (B) High-power view showing columnar epithelium and mitotic activity.

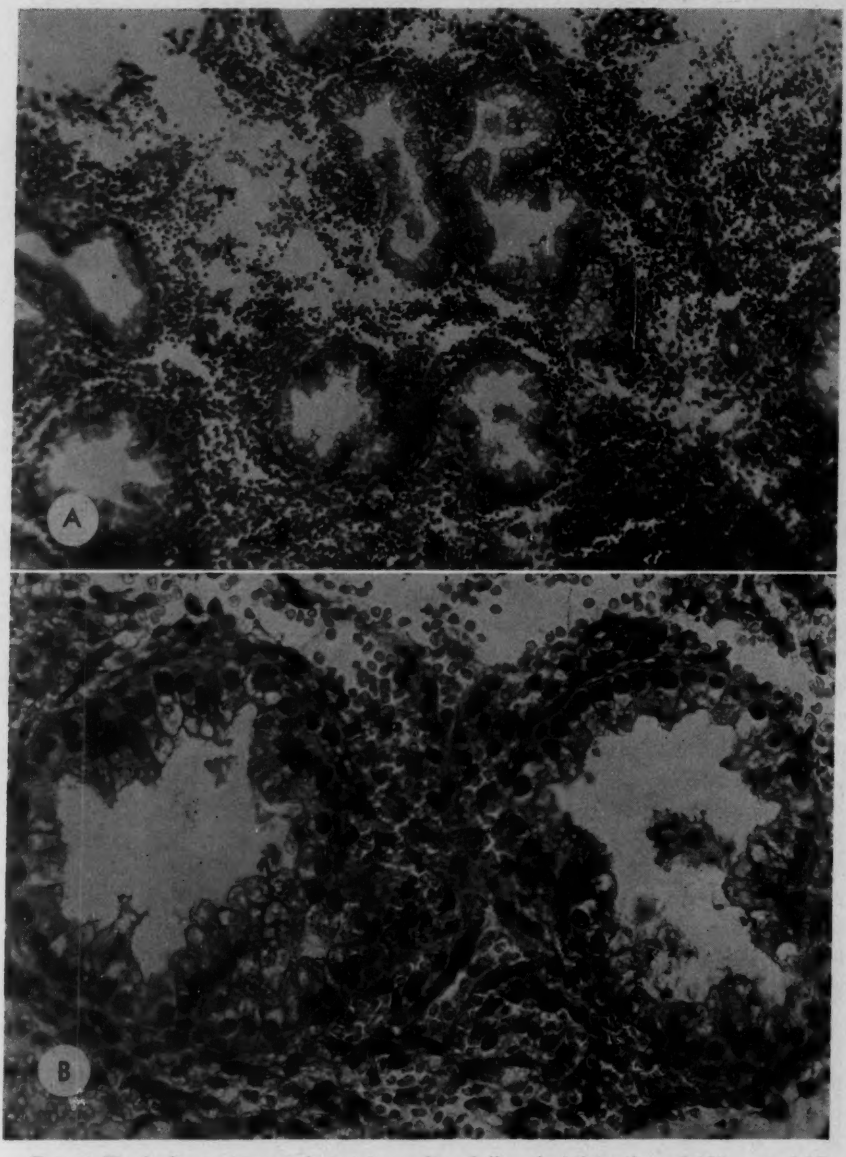

FIG. 2. Typical secretory endometrium after daily administration of 100 mg. of 17-alpha-hydroxyprogesterone acetate (total dosage 1.5 Gm.) (test cycle No. 9)♀ (A) Low-power view showing tortuosity of the glands and edema of the stroma. (B) High-power view showing marked secretory activity of the epithelium, characteristic pale-staining quality, and frayed lumen edge.

reduces fern-like patterns of the cervical mucus, (d) causes a rise of basal body temperature, and (e) induces withdrawal bleeding in castrates. The study was performed on 3 surgically castrated women.

2. Endometrial biopsies showed minimal or mild secretory changes after daily administration of 25 or 50 mg. of 17-α-hydroxyprogesterone acetate over a period of fifteen days. During daily dosages of 75 or 100 mg. over the same period of time, examination of material obtained by endometrial curettage revealed definite to pronounced secretory changes.

3. Cytologic smears showed some luteal changes after daily administration of 75 or 100 mg. of 17-α-hydroxyprogesterone acetate.

4. There was either regression or complete abolition of fern-like patterns of the dried cervical mucus after daily dosages of 50 or 100 mg. of 17-α-hydroxyprogesterone acetate.

5. A rise in basal body temperature was observed in 2 of the 3 castrates after daily administration of 50 or 100 mg. of 17-α-hydroxyprogesterone acetate.

6. Comparative tests performed with anhydrohydroxyprogesterone (pregneninolone, ethinyltestosterone) demonstrated no characteristic secretory changes in the endometrium with dosages of 50 mg., 75 mg. or 100 mg. daily during fifteen days. The oral threshold dosage of anhydrohydroxyprogesterone was determined by administering 150 or 200 mg. daily over a period of fifteen days. 17-α-Hydroxyprogesterone acetate is apparently more than twice as effective as anhydrohydroxyprogesterone.

7. Although the progestational activity of 17-α-hydroxyprogesterone acetate administered orally is definite and seems to be identical with that of progesterone administered parenterally, the activity per milligram is much less. Usually 20 mg. of progesterone given parenterally each day for two weeks induces full progestational change of the endometrium, whereas with the new compound an oral dose of 75 to 100 mg. daily is required.

SUMMARY

A new orally effective substance with progestational activity, 17-α-hydroxyprogesterone acetate, has been compared with anhydrohydroxyprogesterone in regard to its effect on vaginal mucosa, endometrium, cervical mucus and body temperature in 3 surgical castrates. It was found that 17-α-hydroxyprogesterone acetate has a progestational activity which is at least twice that of anhydrohydroxyprogesterone.

Acknowledgment

We wish to express our appreciation to Dr. Edith L. Potter of this department, for her interest and valuable suggestions. The hormones mentioned in this paper were generously supplied by the Upjohn Company, Kalamazoo, Michigan.

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CONGENITAL ADRENOCORTICAL HYPERPLASIA ASSOCIATED WITH HYPERPLASIA OF ABERRANT (INTRATESTICULAR) ADRENAL TISSUE*

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WILKINS (1) has had personal experience with 67 cases of virilizing adrenocortical hyperplasia, 50 in females and 17 in males. Testicular nodules were observed in at least 9 of these cases. In a few the nodule produced enlargement which was detectable clinically, but in most the nodule was first observed at autopsy. It is the purpose of this communication to describe a case of the adrenogenital syndrome produced by congenital adrenocortical hyperplasia with testicular involvement, and to discuss the nature of the lesion found in the testes.

CASE REPORT

History and clinical findings

The patient was a 2½-year-old boy, who had been born at the New Britain General Hospital on July 23, 1952, about four weeks before the expected date of delivery. The mother was a white woman whose blood group was Rh negative. At birth the infant's body weight was 5 pounds, 8 ounces. His blood group was B, Rh positive. The hemoglobin level was 15.4 Gm. per 100 cc., and the red blood cell count was 4.6 million per cu. mm. The red cells exhibited polychromasia and macrocytosis, and there were 10 erythroblasts and 10 normoblasts per 100 white cells. The white cell count was normal. Shortly after birth, the infant was given 140 cc. of blood intravenously and later the blood picture became normal. Since birth the child had had difficulty in feeding, and vomited frequently. Because of vomiting and failure to gain weight, he was transferred to another hospital where a diagnosis of adrenal insufficiency was made.

He was treated with cortisone and physiologic salt solution by hypodermoclysis. Vomiting ceased, and his body weight increased. He was then discharged with instructions to the mother to provide a special diet containing sodium chloride and sodium bicarbonate.

At 15 months of age, the boy was readmitted to that hospital because of vomiting. Examination at this time revealed a large, well developed, muscular male child, in no distress. He had an unusually shaped head covered by a sparse growth of hair. The blood pressure was 90/55 mm. Hg, pulse 110 per minute, respirations 22 per minute and temperature 38° C. The penis was symmetrically enlarged, but the testes were smaller than expected for his age. At this time, the hemoglobin level and red blood cell count were normal. The white blood cell count was 11,700 per cu. mm. The urine was normal. The admission blood chemical values were as follows: carbon dioxide content 18.6 mEq./L.; chloride 93.2 mEq./L.; sodium 132 mEq./L.; potassium 5.5. mEq./L.; nonprotein nitrogen 47.5 mg. per 100 cc.; and sugar 55 mg. per 100 cc. The urinary 17-ketosteroid

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level was recorded as increased. The patient was given 40 mg. of cortisone intramuscularly on the first and second hospital days and 75 mg. of Aureomycin every six hours. He responded to therapy and became afebrile on the third hospital day. Upon discharge, his feeding formula was one quart of cow's milk containing a teaspoonful of sodium chloride and half a teaspoonful of sodium carbonate, and Karo. He also was to receive 20 mg. of cortisone daily. The discharge diagnosis was adrenogenital syndrome with disturbance of salt conservation and androgenic secretion.

At 17 months, the boy weighed 24 pounds and was in good health. The daily dosage of cortisone was changed to 35 mg. At 18 months he weighed 25 pounds and was 82.6 cm. tall. The blood electrolyte levels were found to be within normal limits. At 23 months of age, the body weight was 26½ pounds and the height was 90 cm. At this time he was able to walk and run, and spoke about ten words. He was still receiving cortisone and salt.

At age 30 months, a cough developed with an elevated temperature and occasional vomiting. He was therefore admitted to this hospital. On admission, he was very restless, lethargic and dehydrated. The temperature was 100.2° F. He suddenly expired twelve hours after admission.

Autopsy findings

The autopsy (#55-16) was conducted four hours after death.

Gross examination: The body length was 93 cm. There was no evidence of pubic, axillary or scrotal hair growth. The *penis* was enlarged and without other abnormality. The *heart* weighed 60 Gm. There was whitish thickening of the endocardium of the left atrium, but elsewhere the endocardium was thin and transparent. The valves were normal. In the *lungs* there were multiple foci of fresh hemorrhage. The *spleen* was firm and weighed 40 Gm. The *adrenals* were larger than normal; toegether they weighed 15 Gm. On section, the cortex was composed of a thin yellow outer zone, and a markedly thickened brownish-red inner zone. The medulla was gray and appeared normal. Each *testis* had the usual shape, with a maximum diameter of 1.3 cm. On section, a circumscribed, sharply demarcated oval gray-tan nodule, 0.8 cm. in diameter, could be seen in the hilus of each testis. The *prostate gland* was moderately enlarged. The *lymph nodes* and *thymus gland* were soft and not remarkable. The *pituitary* and *pineal glands* were not altered grossly. There were no gross changes in the remaining organs. A postmortem blood culture yielded *Escherichia coli*.

Microscopic examination. The *endocardium* of the left atrium was thickened as a result of an increase in the elastic tissue fibers and in the amount of collagen. The *lungs* exhibited minimal emphysema, focal areas of atelectasis and bronchiolectasis, with acute bronchiolitis, focal pneumonia and intra-alveolar hemorrhage. There was congestion of the *spleen*, with central necrosis of the lymphoid follicles. Necrosis was also observed in Hassall's corpuscles of the *thymus*. In both *adrenal glands* there were identical changes. In each there was a well-defined broad zone bounded by medulla on the inner margin, and by zona glomerulosa or more often by zona fasciculata on the outer margin (Fig. 1). There were numerous circumscribed adrenocortical nodules. This broad zone was of uniform architecture and consisted of cells arranged in anastomosing cords or columns, one to two cell layers in thickness. Traversing irregularly throughout the parenchyma was a rich network of markedly dilated and congested capillaries. The cells were of two definite types intermingled (Fig. 2). The first type, representing the majority, consisted of relatively large round or polyhedral cells with a distinct cell membrane. They measured from 16 mμ to 26 mμ in greatest diameter. The nuclei were round, eccentrically

placed, vesicular and relatively large, with one or two nucleoli. The cytoplasm was relatively voluminous, pale, granular and acidophilic, with infrequent small vacuoles. There were diffuse basophilic cytoplasmic granules, which in some of the cells were arranged in a well-formed circle surrounding a faint reticular mass. The cells of the second type were similar in shape, but had markedly dense, granular acidophilic cytoplasm and relatively small, hyperchromatic nuclei. There was an area of calcification in the surrounding fibrous stroma. Very rarely, brownish granular pigment was seen in the cytoplasm of these cells. No mitotic figures were noted. Sudanophilic granules were present in the cells of the outer third of the cortex. These were scant in the hyperplastic zone. The entire thickness of the zona reticularis and many areas in the zona fasciculata were replaced by this hyperplastic zone. The medulla was not significantly altered. The adrenocortical nodules were in large part composed of hyperplastic tissue. The nodule noted grossly in each *testis* was slightly lobulated and encapsulated by thin fibrous connective tissue at some points (Fig. 3). Each nodule was in direct continuity with the seminiferous tubules on the one hand and with the rete testis on the other. Each was composed of masses of cells divided into small nests or anastomosing cords (two to three layers of cells in thickness) by a rich network of fine fibrous septa which contained numerous vascular spaces lined by a single layer of endothelial cells (Fig. 4). Some of these spaces were engorged with red blood cells. The shape of the cells varied from round to polyhedral, and measured from 12 mμ to 26 mμ in greatest dimension. The nuclei were relatively small, round and vesicular, with one to two eccentric or central nucleoli. The cytoplasm was pale, granular, acidophilic and rarely vacuolated, with tiny, irregularly shaped, basophilic cytoplasmic granules. The smaller proportion of the cells had a dark, dense, granular acidophilic cytoplasm with a small hyperchromatic nucleus. Reinke's crystalloids and brown cytoplasmic pigment were not identified. Sudan IV-stained preparations failed to demonstrate the presence of fat droplets in these cells. Mitotic figures were absent. There was no active spermatogenesis in the surrounding testicular tissue, and no interstitial cells were seen in the stroma. In addition to the nodules noted on gross examination, there were at least 2 additional circumscribed, partially encapsulated nodules composed of anastomosing columns of hyperplastic adrenocortical cells in the region of the epididymis of one testis (Fig. 5). The *prostate gland* was hyperplastic and there was some secretory activity of the glandular epithelium. In the *pituitary gland* there was definite hyperplasia of the basophilic cells with reduction of acidophilic cells in the anterior lobe. Crooke's cells were present. The *pineal gland* was normal.

DISCUSSION

The case presented here is characteristic of the adrenogenital syndrome resulting fron congenital bilateral adrenocortical hyperplasia. As in many similar previously reported cases (2, 3, 4) there was also mild adrenocortical insufficiency. The first symptom, vomiting, appeared early in the course of the disease. The child's growth was not significantly accelerated—probably the result of the early institution of proper therapy. The genitalia, particularly the penis and the prostate (as later determined at autopsy), were abnormally large. The urinary 17-ketosteroids were elevated, and the serum sodium level was low. Later in the course of the disease a chronic respiratory tract infection developed. Terminally there was an acute exacerbation of this infection, and presumably bacteriemia.

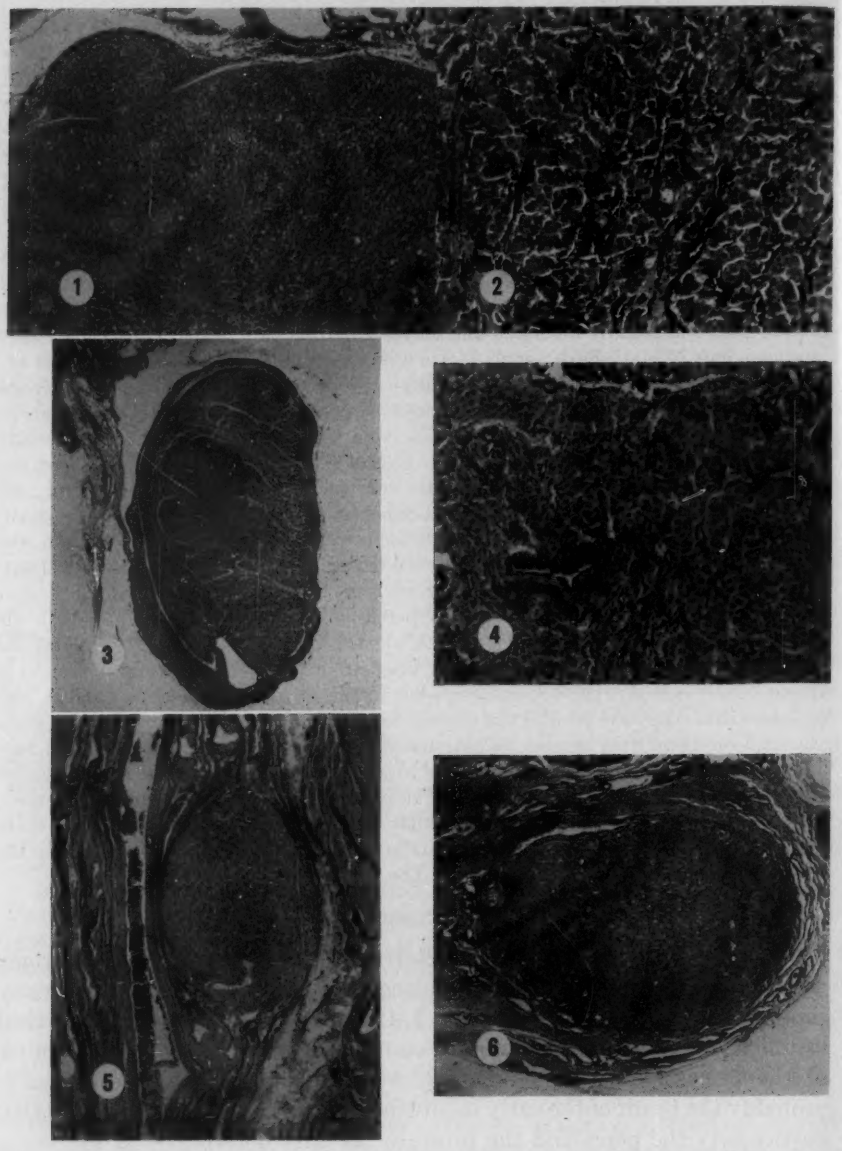

FIG. 1. Adrenal gland showing hyperplasia of the inner cortical zone and a cortical nodule. ×30.

FIG. 2. Adrenal gland showing structural and cellular patterns of the hyperplastic inner cortical zone. ×100.

As the name implies, congenital adrenocortical hyperplasia is believed to result from a defect in the intra-uterine or post-natal development of adrenal tissue. The hyperplasia involves cells of the reticular zone. According to Blackman (5), the adult reticular cells develop from cells of the reticular zone in the fetus which fail to undergo involution. The brown pigment which occurs in the adult zona reticularis cells, and which was in only scanty amounts in the adrenal of the case described here, is not ordinarily present in the first few years of life. In Blackman's (5) series of infant pseudohermaphrodites, this pigment was absent in the cells comprising the hyperplastic reticular zone of the adrenal cortex. These cells are believed to be concerned with the metabolism of the masculinizing sex hormones.

Nodular lesions in the testes are occasionally observed in patients with congenital adrenocortical hyperplasia. Wilkins *et al.* (2), Cohen (3), Thelander (4), Allibone *et al.* (6), and Butler *et al.* (7) have reported cases with testicular nodules, and all considered these nodules to represent aberrant or ectopic adrenal tissue. Landing and Gold (8), however, concluded from their study of 3 cases of adrenocortical hyperplasia in infants with associated testicular nodules, that the testicular lesions represented nodular hyperplasia of the interstitial Leydig cells. In none of these cases were crystalloids of Reinke demonstrated. Because crystalloids of Reinke are usually not present in lesions designated as "interstitial-cell tumors," Karsner (9) raised the question as to whether these tumors are derived from interstitial cells, or from adrenocortical inclusions. The nodules under discussion, when present in the testis, are often bilateral and usually located in the hilus. Aberrant adrenocortical tissue has been found elsewhere in the testis, including its mediastinum (10). Testicular tissue is derived from the undifferentiated gonads on the ventral surface of the mesonephros, the region from which the adrenal cortex arises. This is probably the explanation for the presence of aberrant adrenocortical tissue in the body of the testis.

FIG. 3. Sharply demarcated partially encapsulated nodule in the body of one testis. ×6.

FIG. 4. Detail of intratesticular nodule shown in Figure 3. The cells and structural patterns are similar to those of the hyperplastic adrenocortical zone. Note the rete testes surrounded by the nodular tissue. ×97.

FIG. 5. An extratesticular, partially encapsulated, hyperplastic aberrant adrenocortical nodule in the region of the epididymis of a testis. ×22.

FIG. 6. For comparison—an extratesticular, completely encapsulated aberrant adrenocortical nodule in the region of the epididymis of a testis of a newborn infant (Autopsy #54–154). ×27.

The child reported here had at least two extratesticular islands of ectopic adrenocortical tissue in the region of the epididymis. These were sharply circumscribed, partially encapsulated (Fig. 5), and possessed an appearance characteristic of adrenocortical tissue. For comparison, an extratesticular nodule observed in another case is shown in Figure 6. The question remains as to the identity of the intratesticular nodules which were observed on gross examination. These were well circumscribed and partially encapsulated. The lack of complete encapsulation might perhaps have resulted from active cell growth and hyperplasia. The hyperplastic cells of the adrenal cortex and the cells composing the testicular nodules were identical in all respects. Their appearance and arrangement were indistinguishable. Fatty vacuoles were absent, both in the testicular nodules and in the hyperplastic zone of the adrenal cortex. Crystalloids of Reinke were not present in the testicular lesions. Interstitial cells were not found in the stroma of the unaffected testicular tissue. For all of these reasons it is concluded that the intratesticular nodules were derived from aberrant adrenocortical tissue. The histologic changes in these nodules represent hyperplasia rather than neoplasia, and therefore the lesion is best denominated as "hyperplasia of aberrant adrenocortical tissue."

The pathogenesis of the disease state is uncertain, and the hypothesis of Bartter and co-workers (11) is of interest. These investigators suggested that the disorder results from a primary inability of the adrenal cortex to synthesize S (sugar)-hormone. The production of pituitary corticotropic hormone is thus stimulated. Adrenocortical hyperplasia results, with consequent production of excess adrenal androgen. The evidence of basophilic cell hyperplasia in the pituitary gland of the present case, as in other reported instances of congenital adrenocortical hyperplasia, supports the thesis of an increase in the production of anterior pituitary hormones. Another theory regarding the pathogenesis has been advanced by Albright *et al.* (12), who suggest that the disease results primarily from overproduction of pituitary luteinizing hormone, with secondary changes in the adrenal gland. Neither of these two hypotheses has as yet been verified, but the weight of evidence indicates that the primary lesion is in the adrenal gland, with secondary changes in the pituitary.

SUMMARY

A case of congenital bilateral adrenocortical hyperplasia associated with mild adrenocortical insufficiency and aberrant intratesticular adrenal tissue is presented. The nature of the nodules found in each testis is discussed. It is concluded that these nodules represent hyperplasia of aberrant adrenocortical tissue rather than neoplasia or hyperplasia of the interstitial cells.

Acknowledgment

We wish to express our gratitude to Dr. Orville T. Bailey for assistance in the interpretation of the microscopic findings in the pituitary and pineal glands.

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Letter to the Editor

PROGESTATIONAL ACTIVITY OF CERTAIN 19-NOR STEROIDS. COMPARATIVE ASSAYS IN OOPHORECTOMIZED WOMEN

TO THE EDITOR:

As early as 1953 Tullner and Hertz (1) observed in the rabbit that 19-norprogesterone, subcutaneously administered, was four to eight times more active than progesterone, and that 17-α-ethinyl-19-nortestosterone (norethisterone), orally administered, was about five times more potent than 17-α-ethinyltestosterone (2). The strong progestational activity of norethisterone by the oral route, in women, was first discovered in the United States (3–5), and was later confirmed by European researchers (6, 7).

Progestational activity is not exclusive to the 19-nor derivatives of progesterone and ethisterone. It has been found that 17-α-methyl-19-nortestosterone (normethandrolone or methylestrenolone) has a remarkable progestational effect in the human female (8, 9). This observation was confirmed in the laboratory (10–13) as well as by new clinical observations (6, 7, 14, 15).

Subsequently, 17-α-ethyl-19-nortestosterone (norethandrolone or ethylestrenolone) was found to be a potent progestational steroid in the human female (7, 15, 16) as well as in animals (12, 13).

We have compared the progestational activity of these various 19-nor derivatives in the oophorectomized human female. With this in mind, we determined the threshold dose required for release of a massive glycogen load in the endometrial glands (lucid-zone or subnuclear vacuolization) (9).

Patients

Assays were performed in 3 bilaterally oophorectomized women who received regularly various combinations of sex hormones over a considerable period of time:

1. Patient V.D.H., aged 39, had been surgically castrated elsewhere on February 12, 1948; the indications were unknown. She had been under treatment with sex hormones since December 1953.

2. Patient V.A., aged 57, had undergone a right salpingo-oophorectomy for rupture of an ectopic (tubal) pregnancy in July 1934 and a left salpingo-oophorectomy for the same condition in October 1939. She had been under treatment with sex hormones since the second operation.

TABLE 1

	Dosage (mg./24 hrs.)	Endometrial response*		
		Patient 1	Patient 2	Patient 3
I. *17-α-Methyl-19-nortestosterone (normethandrolone)*				
Sublingually:	25.0	4+	4+	4+
	12.5	4+	4+	4+
	5.0		4+	4+
	2.0	4+		
	1.0		2+	4+
	0.5	3+	2+	4+
	0.3	1+		3+
	0.2	0	0	
	0.1	0	0	0
Intramuscularly:	0.5			4+
II. *17-α-Ethyl-19-nortestosterone (norethandrolone)*				
Sublingually:	25.0	4+	4+	
	2.0	4+	3+	3+
	1.0	4+	1+	
	0.5	1+	0	0
III. *17-α-Ethinyl-19-nortestosterone (norethisterone)*				
Sublingually:	10.0	4+	4+	
	1.0	4+	4+	
	0.2	3+	1+	4+
	0.1	2+	0	0
	0.05	0	0	
IV. *19-Norprogesterone*				
Intramuscularly:	25.0			4+
	2.0			4+
	0.5			3+
	0.1			0
V. *Progesterone*				
Intramuscularly:	5.0			3+
	2.0			0
Sublingually:	150.0		1+	
	100.0	0	0	1+
	50.0		0	
	25.0	0		
VI. *17-α-Ethinyltestosterone (ethisterone)*				
Sublingually:	100.0		3+	
	50.0	1+	1+	3+
	25.0	0	0	0

* For explanation of symbols, see text.

3. Patient D was 39 years old. In 1947 a right salpingo-oophorectomy had been performed for rupture of an ectopic (tubal) pregnancy, and in October 1948, a left salpingo-oophorectomy for a tubo-ovarian abscess. She had been under treatment with sex hormones since October 1949.

It is to be emphasized that, in these 3 women, no variation in endometrial response was observed after similar courses of estrogenic therapy.

Method

A single daily sublingual standardized dose of 50 micrograms of ethinyl estradiol was administered every day for twenty days; in addition, during the last five days, a varied quantity of a progestational steroid was given, either in single or multiple doses. Between each course of treatment there was an interval of eight to ten days, to allow for the possible occurrence of withdrawal bleeding.

On the last day of each course of treatment, an endometrial biopsy was performed, and the various degrees of response were distinguished on the basis of the amount of subnuclear vacuolization in the endometrial glands.

Results

The results are shown in Table 1. The various degrees of subnuclear vacuolization are distinguished as follows:

4+—a large basal vacuole in all the glandular cells;
3+—a basal vacuole in all the glandular cells;
2+—a basal vacuole in several cells of the majority of glandular sections;
1+—a basal vacuole in several cells of a few glandular sections;
0 —no significant basal vacuolization.

CONCLUSIONS

The 19-nor derivatives, norprogesterone, normethandrolone, norethandrolone and norethisterone, exert a strong progestational effect in women. Their respective activities are essentially similar. They are much more active than intramuscularly administered progesterone and many times more potent than ethisterone and sublingually administered progesterone.

Acknowledgments

The author gratefully acknowledges the generous supplies of 17-methyl-19-nortestosterone, 17-ethyl-19-nortestosterone and 17-ethinyl-19-nortestosterone from the Organon Laboratories (Oss, Holland); of 17-ethinyl-19-nortestosterone from the Schering Laboratories (Berlin, Germany); and of 19-norprogesterone from the Roussel Laboratories (Paris, France).

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The Endocrine Society

THE 1958 ANNUAL MEETING

The Fortieth Annual Meeting of The Endocrine Society will be held in the St. Francis Hotel, San Francisco, California, Thursday, Friday and Saturday, *June 19, 20 and 21, 1958.*

THE 1958 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955, Dr. Frederick L. Hisaw in 1956, and Dr. Joseph C. Aub in 1957.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

NINTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE MEDICAL COLLEGE OF GEORGIA

Augusta, Georgia, October 21-25, 1957

The faculty will consist of 24 eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the Assembly.

For further information concerning the program and registration, write to *Dr. Robert B. Greenblatt, Department of Endocrinology, Medical College of Georgia, Augusta, Georgia.* Registration is limited to 100; the tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Bon Air Hotel. Residents and fellows will be admitted for $35.00.

(For Faculty and Program, see June issue)

ALBERT EINSTEIN MEDICAL CENTER—POSTGRADUATE COURSE IN CLINICAL ENDOCRINOLOGY

A Postgraduate Course in Clinical Endocrinology will be conducted at the Albert Einstein Medical Center, Southern Division, Fifth and Reed Streets, Philadelphia 47, Pa., beginning *November 27, 1957*. The course will consist of 39 hours of instruction divided into 13 sessions of 3 hours each. The sessions will be held on consecutive Wednesdays from 2 to 5 P.M. The fee for the course is $60.00. Registration closes on *November 16, 1957*. Information may be obtained from Dr. George I. Blumstein, Chairman, Postgraduate Medicine, Albert Einstein Medical Center Executive Offices, York & Tabor Roads, Philadelphia 41, Pennsylvania.

INTER-SOCIETY CYTOLOGY COUNCIL

The Annual Scientific Meeting of the Inter-Society Cytology Council will be held in the Bon Air Hotel, Augusta, Georgia, *November 14, 15 and 16, 1957*. Paul F. Fletcher, M.D., 634 North Grand Avenue, St. Louis 3, Missouri, *Secretary*.

BOOK REGISTER

Cryptorchism. By CHARLES W. CHARNY, M.D., Associate Attending Urologist, Albert Einstein Medical Center, Southern Division, Philadelphia, Pa. and WILLIAM WOLGIN, M.D., Assistant Urologist at the same institution. This book is an attempt to clarify the present confused state of knowledge regarding the management of undescended testes, based on extensive clinical experience and on a thorough review of the literature. The advantages and disadvantages of hormonal therapy and of orchiopexy are discussed. Conclusions are reached on the basis of testicular biopsy, so that the results of therapy can be more readily appraised in relation to infertility; 140 pages; 28 figures; 1957. Paul B. Hoeber, Inc., New York, N. Y. Price $5.85.

Experiments in Biochemical Research Techniques. By ROBERT W. COWGILL, Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado, and ARTHUR B. PARDEE, Department of Biochemistry and Virus Laboratory, University of California, Berkeley, California. The experiments in this manual are for advanced students and for the more advanced investigator. They illustrate the actual methods used by workers in modern research, including use of radioisotopes, electrophoresis, paper chromatography, gas chromatography, and techniques for enzyme isolation, assay, and intermediary metabolism. A useful guide to procedures and applications and the key literature of modern biochemical research techniques: 188 pages; 21 figures; 16 tables; 1957. John Wiley & Sons, Inc., New York, N. Y. Price $3.50.

Hormonal Regulation of Energy Metabolism. Compiled and edited by LAURANCE W. KINSELL, M.D., Institute for Metabolic Research, Highland-Alameda County Hospital, Oakland, California. This book contains the Proceedings of the Conference on Hormonal Regulation of Energy Metabolism held in 1956, in which many prominent clinicians and medical scientists took part. The opening remarks are by ELLIOTT P. JOSLIN, M.D. It is a critical evaluation of current knowledge and concepts in a field of fundamental importance. The general subjects of discussion are Hormonal Regulation of Carbohydrate Metabolism, Hormonal Regulation of Enzymatic Activity, the Anterior Pituitary in Relation to Energy Metabolism, the Mechanism of the Influence of Pituitary Growth Hormone on Metabolism, the Thyroid in Relation to Energy Metabolism, Insulin, Diabetes and the Insulin Problem, and the Adrenal Cortex and Energy Metabolism; 256 pages; 1957. Charles C Thomas, Publisher, Springfield, Illinois. Price $5.25.

J.A.M.A. Clinical Abstracts of Diagnosis and Treatment (1957). Selected by I. PHILLIPS FROHMAN, M.D. Published with the approval of the BOARD OF TRUSTEES, AMERICAN MEDICAL ASSOCIATION. These abstracts are culled from "Medical Literature Abstracts" which have appeared in the Journal of the American Medical Association during 1957, and are considered to be those which pertain most directly to diagnosis and treatment. They are now organized in a form which was not possible when they were originally published in J.A.M.A., and appear under the headings: Digestive System, Respiratory System, Cardiovascular System, Urinary System, Reproductive System, Endocrine System,

Bones, Muscles, Joints, Connective Tissue, Blood and Lymphatic System, Skin, Nervous System, Eye, Ear, Nose and Throat, Poisonings, Infections, and Miscellaneous Diagnosis and Treatment. The book presents the best of the year's medical literature in greatly condensed form; 564 pages; 1957. Intercontinental Medical Book Corporation with Grune & Stratton, Inc., New York and London. Price $5.50.

Le Tireopatie, Vol. V. Il Gozzo. Studi del 3° Congresso Nazionale sulle malattie della tiroide: Convegno Internazionale sui problemi del gozzo, tenutosi in Torino, il Guigno, 1957. This cloth-bound book, mostly in Italian, contains the papers presented at the National Congress for Study of Diseases of the Thyroid held in Turin, June 4–6, 1957. It is the fifth volume of a series which started in 1951. There are 25 articles covering various aspects of goiter, particularly endemic goiter, and the book should prove of value to those interested in the study of thyroid function and its clinical applications; 838 pages: illustrated; 1957. Per iniziativa e cura dell'Ordine Mauriziano. Tipografia L. Checchini, Torino, Italy.

Meprobamate and Other Agents Used in Mental Disturbances. This is the May 9, 1957 issue of the *Annals of the New York Academy of Sciences* (Vol. 67, Art. 10, pp. 671–894). The Contents are in four parts: I. Chemistry, Pharmacology and Mode of Action of Meprobamate; II. Treatment of Psychoneurotic Conditions; III. Treatment of Psychiatric and Other Conditions with Meprobamate; and IV. Use of Meprobamate in Muscle Spasm. There are 23 papers by various authorities in the fields mentioned, given at a conference held by the New York Academy of Sciences, Section of Biology and Psychology, October 18–19, 1956. Published by the New York Academy of Sciences, New York, N. Y.

Obesity. Its Cause, Classification, and Care. By E. Philip Gelvin, M.D., Associate in Medicine, New York Medical College, Flower and Fifth Avenue Hospital; Physician-in-Charge Obesity Clinic, Metropolitan Hospital, New York, and Thomas H. McGavack, M.D., Professor of Clinical Medicine, New York Medical College, Flower and Fifth Avenue Hospital, New York. The authors state in their preface, "We have no panacea to offer, nothing sensational nor magical. . . . The program for management of obesity as presented in these pages is safe, effective, and consistent with principles of good nutrition, yet simple for the physician to prescribe and convenient for the patient to observe." There are discussions of fat metabolism, hereditary, metabolic and endocrine factors, obesity in childhood, and management by diet, exercise, drugs, anorexigenic agents, endocrine preparations, physiotherapy and surgery; 146 pages; charts and tables; 1957. Paul B. Hoeber, Inc., New York, N. Y. $3.50.

Progress in Gynecology. Volume III. Edited by Joe V. Meigs, M.D., Clinical Professor of Gynecology, Harvard Medical School, Visiting Consulting Surgeon, Massachusetts General Hospital, Visiting Consulting Gynecologist, Vincent Memorial Hospital, Boston, and Somers H. Sturgis, M.D., Clinical Professor of Gynecology, Harvard Medical School, and Surgeon (Gynecology) and Head of Department, Peter Bent Brigham Hospital, Boston, Massachusetts. The 69 contributors are outstanding in the field of gynecology. Volume III does not repeat the articles in previous volumes, but is an attempt to present current concepts in one book. The subject is covered in chapters entitled: Growth and

Physiology, Diagnostic Methods, Functional Disorders, Inter-Relationships of Endocrine Glands, Sterility and Reproduction, Infections, Benign Growths, Malignant Growths, Operative Technics, and Preoperative and Postoperative Care; included in a separate folder is a partial list of "Commercial Preparations of Endocrine Products Used in Gynecology"; 794 pages; numerous tables and illustrations; 1957. Grune & Stratton, Inc., New York, N. Y., and London England. Price $15.50.

Stress: Experimental Psychology: Child Psychiatry. Psychiatric Research Report No. 7 of the American Psychiatric Association, edited by Members of the Committee on Research, 1955–56—J. S. GOTTLIEB, R. A. CLEGHORN, H. R. HIMWICH, N. S. KLINE, W. T. LHAMON and G. SASLOW. Papers presented at the Regional Research Conference held under the joint auspices of the American Psychiatric Association and McGill University Faculty of Medicine, Department of Psychiatry, Montreal, P.Q., Canada, November 18–19, 1955, concerning adverse responses of mammals to deleterious changes in their environment, and a consideration of symptom mechanisms. The studies involved biochemical, experimental physiological and clinical approaches to problems in psychiatry; 88 pages; 4 figures; 1957. American Psychiatric Association, Washington 6, D.C. Price $2.00.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Office, 16 West 46th Street, New York 36, N. Y.

Neuropharmacology. Third Conference, May 21–23, 1956, Princeton, N. J. Edited by HAROLD A. ABRAMSON, M.D., Research Psychiatrist, The Biological Laboratory, Cold Spring Harbor; Consultant in Research Psychiatry, State Hospital, Central Islip, New York. Ten articles with discussions cover the following subjects: Blocking of the LSD-25 Reaction in Siamese Fighting Fish, Effect of LSD-25 on Snails, Production and Control of Alcoholic Cravings in Rats, Stress Situations in Animals and the Nature of Conflict, Action of Alcohol on the Central Nervous System, Measurement of Subjective Responses, Effects of Psychomimetic Drugs in Animals and Man, Origin of Cortical Surface Potentials, Serotonin and Norepinephrine as Antagonistic Chemical Mediators Regulating the Central Autonomic Nervous System, and Brain Response to Drugs Mapped through Self-stimulation; 381 pages; 133 figures; 20 tables; 1957. Price $4.50.

Physiology of Prematurity. First Conference, March 21–23, 1956, Princeton, N. J. Edited by JONATHAN T. LANMAN, M.D., Department of Pediatrics, New York University-Bellevue Medical Center, New York, N. Y. There are two main sections, with discussions: 1) Fetal-Maternal Endocrinology in Late Pregnancy, and 2) The Fetal and Placental Circulation in Late Pregnancy; 151 pages; 42 figures; 5 tables; 1957. Price $3.25.